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Male indicator traits to improve female reproductive performance

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Abstract

Prior to the Co-operative Research Centre for Beef Genetic Technologies (Beef CRC), little genetic information on bovine male reproductive traits and their associations with components of female reproduction was available. This study was initiated to identify male indicator traits to improve male and female progeny reproductive performance. The genetic control of traditional and novel measures of male reproductive performance and their genetic correlations with critically important female traits, including age at puberty, lactation anoestrous and traits associated with female lifetime reproductive performance were estimated. This project demonstrated that male traits can be used to indirectly improve reproductive performance (male and female) in northern Australia herds. It was conservatively estimated that an EBV for sperm motility in Brahman cattle may lift lifetime weaning percentage by 6% in 10 years. However, sperm motility was identified as a useful trait in this project through the assessment made by a limited number of highly skilled technical people and the precision was good. If this trait is to be used widely throughout the industry as a predictor of male reproductive performance, additional training and accreditation will be required by the veterinary profession. Further, it may be better to use a suite of semen quality traits in the development of an EBV for male reproductive performance.

Executive summary

Reproduction rate is one of the main drivers of herd productivity and profitability and substantial scope exists to improve the gross value of northern beef production. The main factors affecting herd reproductive efficiency in northern Australian herds relate to delays in breeding females returning to oestrous after calving (lactational anoestrous / prolonged post-partum anoestrus interval) and age at puberty in *Bos indicus* breeds. As weaning rates of 50% or less are not uncommon in some northern herds, there is an urgent need to optimise beef production efficiency through the identification and implementation of efficient reproduction strategies.

The objective of this study was to define the genetic control of traditional and novel measures of male reproductive performance and their genetic correlations with critically important female traits, including age at puberty, post-partum anoestrous and traits associated with female lifetime reproductive performance. New traits in tropically adapted males to indirectly improve reproductive performance of male and female progeny were identified.

The Beef CRC project generated 4063 male progeny weaned from Brahman and Tropical Composite breeds over 7 years (3648 progeny evaluated to 24 months of age). The data collected allowed the estimation of breed-specific heritabilities and genetic correlations for male fertility traits and were subsequently used to estimate genetic correlations with female reproduction traits using dam/son relationships. The progeny were generated by natural mating of the cows involved in the female reproduction experiment established in Beef CRC II. Forty Brahman and Tropical Composite sires were used across years and locations to generate genetic linkage. Progeny were bred on Department of Employment, Economic Development and Innovation (DEEDI) Research Stations (Brian Pastures, Belmont, Toorak, Brigalow and Swan's Lagoon) and CSIRO Belmont Research Station. Bull progeny were evaluated for a range of traits at Brigalow and Belmont Research Stations from approximately 6 months of age (weaning) until 24 months of age.

The measurements and samples collected included the blood hormones pre-pubertal Gonadotrophin Releasing Hormone (GnRH)-stimulated luteinising hormone (LH); pre-pubertal inhibin; and pre-pubertal insulin-like growth factor-1 (IGF-1). A bull breeding soundness evaluation (BBSE) was employed both for its intrinsic value and also for evaluation of the genetic associations between pre-pubertal and post-pubertal traits. BBSE measurements were conducted at 12, 18 and 24 months of age and were based on the recommendations of the Australian Cattle Veterinarians (ACV). Key components of this BBSE included a physical examination of an animal's conformation/structure and reproductive organs (scrotal circumference and testicular tone). At each BBSE, samples of sera (evaluated 11β-Hydroxysteroid dehydrogenase and its association with cortisol and cortisone), seminal fluid (investigated seminal plasma proteins by electrophoresis) and semen (evaluated semen quality - specifically percent morphologically normal sperm (PNS)) were collected for either immediate crush side evaluation or stored for future evaluation. Other traits measured included a comprehensive range of measures of production, e.g., live weight, fatness, eye muscle area (EMA) and hip height.

This project found:
1. New male traits that are heritable and genetically associated with scrotal circumference or female reproductive traits;
2. No antagonisms between these male reproductive traits and other production traits;

3. Potential to use male traits for indirect selection to improve both male and female reproductive performance in Northern Australia herds; (also reported by David Johnston in the Beef CRC 'Early predictors of lifetime female reproductive performance', MLA Final Report, NBP.363);
4. Genetic parameter estimates that can be age specific and can help improve recording protocols for genetic evaluation; and
5. Value in seedstock producers conducting BBSE for current herd fertility (as per current recommendations), and a new role for helping improve the future fertility of the herd (through improving the genetic merit for reproduction of progeny).

Further, using the genetic parameter estimates generated from this project, the prediction of the response to selection for sperm motility alone indicated that a 6% increase in lifetime weaning rate may be achieved in Brahmans over 10 years. These results can be used to underpin new genetic evaluation and multi-stage selection strategies to identify young replacement bulls with superior genetic merit for herd reproduction traits. This result and the subsequent ability to increase the selection pressure on bulls will result in increased rates of genetic improvement for herd reproductive performance and enable increases in herd profitability to be achieved in northern Australia.

Recommendations from this project include:-

1. Distil all the information from the Beef CRC, MLA funded projects NBP.361, 363 and 364 and focus on the implementation and adoption of key messages and outputs that will deliver improved reproductive performance by genetic selection.
2. Examine the practically important messages to emanate from this project with regards to 'take home' messages for every commercial bull breeder in northern Australia irrespective of their previous level of herd recording. .
3. The need for the development and implementation of a long term project to evaluate and demonstrate the economic benefits of selection for Lifetime Weaning Rate over a 10 year period in Brahman, Droughtmaster and Santa Gertrudis breeds in northern Australia.

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1 Background

Reproduction rate is one of the main drivers of profitability in the northern beef industry and weaning rates of less than 50% are commonly reported for many northern Australian beef cattle herds[1] [2] . Developing cost effective strategies to improve reproduction performance in the breeder herd is therefore a logical approach to help address the factors identified above. While genetic improvement is slow, the results are cumulative and can be achieved at little or no additional cost to the enterprise.

However, in the past, and as a consequence of these low weaning rates in many northern Australian beef enterprises, direct selection for cow fertility could only be applied at low intensities, and relatively late in life when the cows had one or two calving records[3, 4]**.** Although the Days to Calving EBV's had been developed as a selection trait when purchasing bulls, uptake in northern herds has been very low and currently only four Brahman BREEDPLAN seedstock herds record this trait (C. Duff pers comm). In addition, the heritability of female reproduction in tropical beef genotypes was thought to be low as is the case in *Bos taurus* genotypes[5,6] and therefore many producers believed that they could make very little improvement to their herd fertility through genetic selection. However, bulls are responsible for a large component of the overall genetic gain in a beef herd due to the higher selection intensities that can be achieved relative to those in females. Therefore, herd reproductive performance could be improved if male traits were identified that are genetically correlated to female reproductive traits and these male traits are able to be measured early-in-life and at low cost[4].

Prior to the commencement of the current Co-operative Research Centre for Beef Genetic Technologies (Beef CRC), little genetic information on bovine male reproductive traits and their associations with components of female reproduction rate was available apart from scrotal size[7]. Age-corrected scrotal circumference was consistently reported to be a useful method of assessing reproductive function in bulls because it was highly heritable and there was a favourable relationship with a number of sperm traits[7] and fertility[8]. Apart from a few studies in tropically adapted genotypes[9,10,11]**,** the majority of research in this area has involved *Bos taurus* beef and dairy cattle genotypes. Further, as the hormonal control of reproductive function is similar in the two sexes, male and female reproductive traits should be closely correlated genetically[12]. Hence, a number of studies have focused on investigating endocrine factors as predictors of reproductive performance. These include the investigation of –

1. Gonadotrophin Releasing Hormone (GnRH-stimulated testosterone response in Hereford-Shorthorn and ¼ Africander ¼ Brahman x ½ Hereford-Shorthorn bulls

[1]Burns et al. (2010) *Animal Reproduction Science* **122** (1-2), 1-22.
[2] McCosker et al. (2011) Proceedings of the Northern Beef Research Update Conference, 19-23.
[3]Mackinnon et al. (1989) *Australian Journal of Agricultural Research,* **40**, 1085–1097.
[4]Burns et al (2011) *Reproduction in Domestic Animals* **46**, 534-553.
[5] Davenport et al. (1965). *Journal of Animal Science,* **24**, 434-437.
[6] Dearborn et al. (1973) *Journal of Animal Science* **, 36**, 1032-1040.
[7]Brinks et al. (1978) Proceedings, Western Section, American Society of Animal Science 29, 28–30.
[8]Mackinnon et al. (1990) *Animal Production* 50, 417–424.
[9]Meyer et al. (1990) *Livestock Production Science,* **25**, 15-30.
[10]Meyer et al. (1991) *Journal of Animal Science*, **69**, 3533-3543.
[11] Burrow HM (2001) *Livestock Production Science* **70**, 213-233
[12]Land RB (1973) Nature 241, 208.

and its relationship to pregnancy rate in similar female genotypes (17-29 months of age)[13];
2. GnRH-stimulated Luteinising Hormone (LH) response in British, ½ Africander x ½ Hereford-Shorthorn, ¼ Africander ¼ Brahman x ½ Hereford-Shorthorn, Brahman cross and high grade Brahman bulls and its relationship to pregnancy rate (16-30 months of age)[14];
3. Age of puberty in British, ½ Africander x ½ Hereford-Shorthorn, ¼ Africander ¼ Brahman x ½ Hereford-Shorthorn, Brahman cross and high grade Brahman heifers through radioimmunoassay of progesterone levels and its relationship with age of puberty in the same bull genotypes as determined by scrotal circumference, semen production and examination, sperm motility and a modified libido/serving capacity assessment (16-30 months of age)[15];
4. GnRH-stimulated testosterone response in ½ Africander x ½ Hereford-Shorthorn and ¼ Africander ¼ Brahman x ½ Hereford-Shorthorn bulls and its relationship to scrotal circumference with the objective of improving both male and female fertility (9-18 months of age)[16];
5. Pre-pubertal Hereford bulls to ascertain LH, follicle stimulating hormone (FSH) and testosterone blood levels to determine their relationship with age of puberty (2 to 50 weeks of age)[17];
6. Basal and GnRH-stimulated concentrations of FSH, LH, testosterone, androstenedione and oestradiol to determine their relationships with testis size and number of Sertoli and germ cells in Angus bulls (2 to 12 months of age)[18];
7. The growing body of evidence suggesting that the majority of endocrine factors associated with reproduction in dairy cattle are a result of differential gene expression in the hypothalamus, pituitary, ovary and uterus[19];
8. Basal and GnRH-stimulated concentrations of FSH, LH and testosterone to determine their relationships with testis size, semen production and quality (morphology) and age of puberty in Hereford x Charolais bulls (2 to 48 weeks of age)[20];
9. IGF-1 levels in pre-pubertal Angus bulls to determine its relationship with adult scrotal circumference and sperm motility and the age at first calf of female progeny and calving rate (5-8 months of age)[21]; and
10. The relationship between Luteinising Hormone Releasing Hormone (LHRH)-stimulated LH response and age of puberty in Hereford x Charolais bulls (1-5 months of age)[22].

Despite these studies, limited genetic information on early-in-life predictor traits of lifetime male and female reproductive performance in tropical beef breeds remains a constraint to selection for improved fertility. Finally, while new sperm morphological traits, particularly percent morphologically normal sperm (PNS) in an ejaculate, have been identified as important phenotypic predictors of calf output of tropically adapted bulls in multiple-sire mated herds in northern Australia (MLA funded Bull Power Project, NAP3.117)[23], the link between PNS and female reproductive performance has not been established. This male trait is typically measured at two years of age. If PNS or other early-in-life predictors of fertility in males could be identified, and these

[13] Post et al. (1987) GnRH. *Theriogenology* **27**, 317–32.
[14] Perry et al. (1990a) *Australian Veterinary Journal* **67**, 13–16.
[15] Perry et al. (1990b) *Australian Veterinary Journal* **67**, 4–5.
[16] Mackinnon et al. (1991) *Livestock Production Science* **29**, 297–309.
[17] Evans et al. (1995) *Theriogenology* **43**, 569-578.
[18] Moura et al. (1997) *Journal of Reproduction and Fertility* **111**, 183–190.
[19] Darwash et al. (1999) *Animal Science* **68**, 333–347.
[20] Aravindakshan et al. (2000) *Theriogenology* **54**, 339–354.
[21] Yilmaz et al. (2004) *Journal Animal Science* **82**, 2285-2292.
[22] Bagu et al. (2006) *Theriogenology* **66**, 37–944.
[23] Holroyd et al. (2002a) *Animal Reproduction Science* **71**, 67–79.

were positively associated with fertility in their daughters, this would greatly improve the efficiency of selection of sires for reproductive performance.

There are both genetic and economic advantages in identifying new traits in the male to indirectly improve the reproductive performance of both male and female relatives. Identifying early-in-life predictors of an individual's fertility could assist to increase the selection intensity placed on bulls at younger ages and thereby substantially reduce the number of replacement sires that need to be kept. Further, the identification of new traits that are related to the fertility of a bull's progeny would also allow opportunities to increase rates of genetic improvement for all traits and significantly increase the impact of using genetically superior bulls in commercial herds in northern Australia.

To help improve female reproductive performance in tropical beef cattle genotypes in northern Australia, this study within the Beef CRC was initiated to identify and evaluate male indicator traits to improve male and female progeny reproductive performance. The study commenced in April 2005 and was completed by June 2012, and has the broader objective of estimating genotype-specific Brahman and Tropical Composite heritabilities and genetic correlations for reproduction.

2 Project objectives

1. Identify early-in-life predictors of fertility both phenotypically (bull fertility as reflected by improved calf output) and genetically (the fertility of a bull's female and male progeny, such as age at puberty).
2. Improve lifetime reproductive performance of females.

3 Methodology

3.1 Project Design

The wider Beef CRC III Project generated 4063 weaned male progeny over 7 years, with records collected to 24 months of age (3648 progeny evaluated to 24 months of age) from Brahman (BRAH) and Tropical Composite genotypes (TCOMP). This allowed the estimation of breed-specific heritabilities and genetic correlations for male reproduction traits, and genetic correlations with female reproduction traits using dam/son relationships. The progeny were generated by natural mating from the cows involved in the female reproduction experiment established in Beef CRC II (Project 2.3 - Links between the Genetics of Beef Quality and Components of Herd Profitability in northern Australia) which has been previously described[24],[25]. Approximately 80-100 industry representative sires per genotype were initially planned to be used to generate approximately 20 male progeny per sire. However, the actual numbers of progeny generated and sires used differed to those forecast due to availability of suitable sires, variation created by the bull to cow mating ratios in the multi-sire natural mating groups, differences in sex ratios and differences in weaning rates across locations and genotypes. Table 1 summarises the actual sire and bull progeny distributions in the dataset for those young bulls with at least a weaning weight record. Genetic linkages across years and location were a key element of the design for estimation of genetic parameters.

[24] Barwick et al. (2009*a*) *Animal Production Science* **49**, 351-366
[25] Burns et al. (2013) *Animal Production Science* **53**, 87–100

Table 1. Numbers of all male progeny weaned and sire distributions

Genotype	Number of progeny weaned	Number of sires	Approximate progeny per sire (range)	Number of sires ≥ 20 progeny	Number of link sires[1]	% Progeny by link sires
BRAH[2]	1639	60	27 (3-75)	37	13	36
TCOMP[2]	2424	76	32 (2-85)	47	27	48
Total	**4063**	**136**	**30 (2-85)**	**84**	**40**	**43**

[1]Sires with male progeny at more than one birth location
[2]Brahman (BRAH) and Tropical Composite (TCOMP) genotypes

3.2 Location

3.2.1 Location, animals, husbandry and management

A detailed description of the location, animals and husbandry and management employed have been described by Burns et al. 2013[25].

3.2.1.1 Generation of male progeny by year, breed/genotype, birth location and post-weaning location

The male progeny were sired by tropically adapted Brahman and Tropical Composite industry representative bulls used in research herds located on Qld Department of Employment, Economic Development and Innovation (now Qld Department of Agriculture, Fisheries and Forestry) and CSIRO research stations in Queensland. The research herd locations and breeds used to generate the male experimental progeny included –

(i) Brian Pastures Research Station, latitude 25.66 °S longitude 151.75 °E, located near Gayndah (Tropical Composite);
(ii) Swans Lagoon Beef Cattle Research Station, latitude 19.62 °S longitude 147.38 °E, located near Millaroo *via* Ayr (Brahman);
(iii) Toorak Research Station, latitude 21.03 °S longitude 141.80 °E, located near Julia Creek (Brahman and Tropical Composite);
(iv) Brigalow Research Station, latitude 24.84 °S longitude 149.80 °E, located near Theodore (Tropical Composite) which was used as a temporary site to manage a proportion of the Brian Pastures and Toorak breeding female herd during severe drought conditions; and
(v) The CSIRO Belmont Research Station, latitude 23.22 °S longitude 150.38 °E, located near Rockhampton (Brahman and Tropical Composite).

The breeding females (generation 1) located at these sites were intensively measured for early growth[26], age at puberty[27] and adaptation[28]. The cows consisted of two breeds, that is, Brahman (1027 breeding females) and Tropical Composite (1132 breeding females). The Tropical Composite was comprised of genotypes derived 50% from tropically adapted (50% *Bos indicus*, African Sanga or other tropically adapted *Bos taurus* genotypes) and 50% from non-tropically adapted *Bos taurus* genotypes[24].

[26]Barwick et al. (2009b) *Animal Production Science* **49**, 367-382.
[27]Johnston et al. 2009) *Animal Production Science* **49**, 399–412.
[28]Prayaga et al. (2009) *Animal Production Science* **49**, 413-425.

All male progeny (generation 2) produced from the cows described above were used in the project. These animals were born between 2003 - 2010 (2004 branded/weaned progeny, born in 2003 and produced prior to official start of the Beef CRC (~ April, 2005)) and were sired by industry bulls. The number of male progeny by year, breed/genotype, birth location and post-weaning location are reported in Table 2. Sires were chosen that were not closely related to the genetics of the cows and preferably had BREEDPLAN EBVs for reproduction traits (e.g., scrotal size or days to calving).

Table 2. Distribution of young bulls by pre – and post-weaning location, genotype and birth year

Birth to weaning	Weaning to 24 months[1]	2004	2005	2006	2007	2008	2009	2010	Total
Brahman									
Belmont	Belmont	47	103	124	68	84	74	47	547
Belmont	Brigalow	0	0	0	42	19	0	0	61
Swan's Lagoon	Brigalow	44	109	96	150	127	114	49	689
Toorak	Brigalow	19	24	46	29	51	33	13	215
Total		**110**	**236**	**266**	**289**	**281**	**221**	**109**	**1512**
Tropical Composite									
Belmont	Belmont	42	105	101	83	61	84	48	524
Belmont	Brigalow	0	0	0	0	20	0	0	20
Brigalow	Brigalow	0	0	57	62	72	0	0	191
Brian Pastures	Brigalow	72	176	149	195	84	189	147	1012
Toorak	Brigalow	58	79	72	64	110	113	58	554
		172	**360**	**379**	**404**	**347**	**386**	**253**	**2301**
Crossbred[2]									
Belmont	Belmont	**0**	**0**	**0**	**69**	**68**	**60**	**53**	**250**
Total		**282**	**596**	**645**	**762**	**696**	**667**	**415**	**4063**

[1]4063 male progeny weaned over 7 years (2004 to 2010 cohorts) and 3648 male progeny with records collected to 24 months of age (2004 to 2009 cohorts)
[2]Crossbred = Tropical sire X Brahman or *vice versa*.

Cows were exposed in large multiple sire groups (3% bulls) for 12 weeks. Sire parentage was determined by DNA fingerprinting[29] after DNA was extracted from a tail hair sample at birth or from blood collected at branding (approximately 3-4 months of age). DNA collected at this time was also stored for future genome wide association studies.

Mating times at the research sites were –
(i) Brian Pastures Research Station - November-February;
(ii) Belmont, Toorak (and Brigalow Research Station (when required)) - December-March; and
(iii) Swans Lagoon Beef Cattle Research Station - January-April.

[29]Vankan DM (2005) The University of Queensland Animal Genetics Laboratory Factsheet. *http://www.uq.edu.au/ animalgeneticslab/docs/DNA-Parentage Testing Factsheet.pdf*.

Bull progeny weaned comprised a total of 4063 animals generated from seven cohorts (i.e., location and year – 2004 to 2010 cohorts). Data collection on the seventh cohort was completed at weaning (415 x 2010 male progeny) while the data collection on the remaining cohorts was completed at 24 months of age (3648 male progeny - 2004 to 2009 cohorts). In addition, 250 crossbred male animals were born at Belmont Research Station and resulted from the mixed mating of the Brahman and Tropical Composite breeds at that location. Data from the crossbreds were grouped by sire genotype and information on all young bulls sired by Brahman sires was summarised separately to those sired by Tropical Composite sires. Forty Brahman and Tropical Composite sires were used across years and locations to generate genetic linkage.

3.2.1.2 Location and environment of animals post-weaning

At weaning each year, the male progeny from Swans Lagoon, Toorak and Brian Pastures Research Stations were relocated to Brigalow Research Station, while those animals born at Belmont Research Station remained at this site except for 42 Brahman (2007) and 19 Brahman and 20 Tropical Composite (2008) bull calves that were transferred to Brigalow Research Station after weaning (Table 2). All male progeny were evaluated at these two sites for an extensive range of measurements and traits from ~6 months to ~24 months of age. Some blood hormonal measurements/traits were evaluated on these animals at ~4months of age at their respective birth locations.

The post-weaning production system environments of Brigalow and Belmont Research Stations are described below. The long-term climatic parameters measured at Brigalow and Belmont Research Stations are presented in Table 3.

Brigalow Research Station is located 190 km south-west of Rockhampton in the Brigalow belt of central Queensland. On average, about 56% of annual rainfall falls during the November–February period (Table 3.). The experimental animals in this study grazed mainly improved pastures which included green panic (*Panicum maximum* var*. trichoglume*), buffel (*Cenchrus ciliaris*) and rhodes (*Chloris gayana*) grasses, some Fitzroy stylo (*Stylosanthes scabra cv*. Fitzroy) and Seca stylo (*Stylosanthes scabra* cv.Seca)[30]. The stocking rate at this location was 0.45 AE/ha (450 kg per adult equivalent) allowing animals to achieve live weight gains of 0.5-0.75 kg/day over a 7–8 month period (October–November to April–May). Brigalow is a moderately stressful production system environment for cattle due to the high temperatures and parasite burdens experienced in the wet summer months and poorer pasture quality in the dry winter months[30]. Supplementation with a protein meal or a urea and protein meal based dry lick was supplied if required during the dry winter months. The cattle tick (*Boophilus microplus*) which is endemic, gastro-intestinal helminths (*Haemonchus placei, Cooperia* spp., *Trichostrongylus axei* and *Oesphagostomum radiatum*), high ambient temperatures[31] and Bovine Infectious Keratoconjunctivitis (BIK)[32] are the other main constraints to animal production. Buffalo fly (*Haematobia irritans exigua*) has not been considered a problem, as large population numbers are evident for only a few weeks of each year[30]. Occasional severe outbreaks of bovine ephemeral fever occur[30].

Belmont Research Station is located 25 km north of Rockhampton and 40 km from the east coast of Queensland in central Queensland. On average, 61% of mean

[30]Burns et al. (1997) *Australian Journal of Experimental Agriculture* **37**, 399-405
[31]Burns et al. (1986) *Proceedings of the Australian Society of Animal Production* **16**, 163–166.
[32]Burns et al. (1988) *Proceedings of the Australian Society of Animal Production* **17**, 150–153.

annual rainfall occurs between November and February (Table 3). The stocking rate at this site was 0.36 AE/ha supporting similar annual live weight gains to those recorded at Brigalow Research Station[33],[34]. The environment at Belmont Research Station is also moderately stressful for cattle due to the high temperatures and parasite burdens experienced in the wet summer months and poor pasture quality in the dry winter months[33,34]. During the period of low nutrition in winter, cattle are maintained on a mixture of improved and native pastures. A dry lick urea based supplement or whole cottonseed was provided when required. The cattle tick (*Boophilus microplus*), gastro-intestinal helminths (*Haemonchus placei, Cooperia* spp., *Trichostrongylus axei* and *Oesphagostomum radiatum*), high ambient temperatures and humidity and exposure to diseases such as BIK and occasional outbreaks of Bovine Ephemeral Fever have been reported as the other main constraints to animal production[33,34]. Similar to Brigalow Research Station, buffalo fly (*Haematobia irritans exigua*) has not been considered a major parasite problem[34].

Table 3. Long-term climatic parameters for bull post-weaning evaluation sites

Location	Average Maximum Temperature (°C)	Average Minimum Temperature (°C)	Mean Rainfall (mm)	Relative Humidity (9.00am)
Brigalow Research Station				
1968-2011				
November – February	33	20	395	64
March – June	27	13	165	66
July – October	26	10	155	62
Belmont Research Station				
1939-2012				
November – February	32	21	433	68
March – June	27	16	213	72
July – October	26	13	114	49

Source - Bureau of Meteorology.

3.2.1.3 Husbandry and management

At each site, date of birth, calf sex and dam identification number were recorded. After a 2-week weaner training period each year, the bull calves were allocated to site and transported as required (Table 2.). From weaning to the conclusion of data recording at 24 months of age, all animals in the same birth-year cohort were managed as a single group at Brigalow and Belmont Research Stations. Bulls were mustered for measurements at 3-monthly intervals between weaning and when cohort average age was approximately 24 months of age.

Management of progeny followed accepted industry husbandry practices and included –

[33] Anon (1976) CSIRO Australia, Division of Animal Production, Rockhampton.
[34] Turner HG (1982) *Animal Production* **35**, 401-412.

(i) Branding (approximately 3-4 months of age in January-March). All progeny were scored for horned, scurred or polled status (~3 months of age) and those that were not polled were dehorned using either a dehorning knife or a scoop dehorning device, which was dependent on the size of the horn growth; all animals were fire-branded.
(ii) Weaning at approximately 6 months of age in April-June.
(iii) Vaccination with 5 in 1 vaccine against clostridial diseases (*Clostridium tetani, Cl. perfringens* type D*, Cl. novyi* type B*, Cl. chauvoei and Cl. septicum*) at branding with boosters at weaning and annually; long-acting botulism vaccination (*Cl. botulinum* types C and D) at branding; Trivalent (3-germ) tick fever vaccine to protect against tick fever organisms (*Babesia bovis, Babesia bigemina and Anaplasma marginale*) carried by the cattle tick *Boophilus microplus*; bovine ephemeral fever (3-day sickness) vaccine four weeks apart in August-September of the weaning year with a booster in August of the following year.
(iv) Supplementation with protein meal or a urea-based dry lick delivering ~200g crude protein equivalent daily per bull during the dry winter months.

3.2.2 Measurements and phenotypes recorded

Detailed descriptions of the phenotypes measured/recorded in this project have been adapted from a recent publication[25] and the following describes the process and prioritisation of the final suite of traits/phenotypes collected.

3.2.2.1 Selection of Traits to be measured.

Shortly after the commencement of the Beef CRC, feedback from the Beef CRC Scientific Review Committee, 7 November 2005, requested that the Project Team 'give consideration to reducing some of the traditional bull reproductive measurements and replace them with novel parameters that might be more valuable as predictors of male reproductive performance, particularly if these could be measured in the younger (less than 2 years of age) animal'.

To address this recommendation, a strategy was developed to progress the identification of potential early-in-life predictors of reproductive performance of male and female progeny and included –
(i) Establishment of criteria for selection of traditional and candidate traits;
(ii) Development of a methodology for the tabulation of potential phenotypes that define male and female reproductive function; and
(iii) Production of a systematic scientific review of early-in-life predictors of male and female fertility to underpin the proposed study.

A literature review was conducted to assess the potential of a number of novel traits and the following measures were recommended for inclusion in the study[35] [36] -
(i) GnRH-stimulated LH (see Plate 1.);
(ii) Pre-pubertal inhibin (see Plate 1.);
(iii) Pre-pubertal leptin;
(iv) Pre-pubertal Insulin-like Growth Factor-1 (IGF-1) (see Plate 2.);
(v) Seminal proteins by electrophoresis;
(vi) 11β-Hydroxysteroid dehydrogenase; and
(vii) Bone morphogenetic protein receptor-1B gene.

[35]Burns et al. (2005) *Review for Beef CRC Scientific Review Committee*, 7 November 2005.
[36]Burns et al. (2011a) *Reproduction in Domestic Animals* **46**, 534-553.

The review was refereed by the internationally recognised bovine reproduction specialist, Professor Heriberto Rodriquez-Martinez of the Swedish University of Agricultural Sciences, who supported the review's recommendations[35] with some minor modifications. Professor Martinez also recommended that a full Bull Breeding Soundness Evaluation (BBSE) be maintained, both for its intrinsic value and also for evaluation of the genetic linkages between these pre-pubertal and post-pubertal markers. Subsequently, analytes (i), (ii) and (iv) were flagged for evaluation in this project (MLA B.NBP 0361 - Male indicator traits to improve female reproductive performance).

In addition to analyte (i), analytes (v) and (vi) were chosen for evaluation in the PhD study of Jessica Crisp (2007-2012) (Team Member) - 'Causes of variation in fertility of physically normal tropically adapted bulls' (MLA Postgraduate Scholarship/Study Award and Beef CRC top-up scholarship). The seminal plasma protein research activity, i.e., 'Seminal plasma proteins as early life indicators of male and female reproductive performance' Project was funded by MLA through a second Project (Project B.NBP.0507). Preliminary results are presented here. Another sub-project of the PhD studied analyte (vi), '11β-Hydroxysteroid dehydrogenase and its association with cortisol and cortisone levels in two year old tropically adapted beef bulls and their relationship to reproductive traits' and its research activities were funded through a University of Queensland research grant. Progress results are also presented in this report.

Further, BBSE measurements at 12, 18 and 24 months were retained based on the recommendation by Professor Heriberto Rodriquez-Martinez. A BBSE was conducted based on the recommendations of the ACV[37],[38]. Key components of this BBSE included a physical examination of the head and eyes, legs, feet and reproductive organs (conformation and scrotal traits (scrotal circumference and testicular tone)). At each BBSE, samples of sera (11β-Hydroxysteroid dehydrogenase and its association with cortisol and cortisone), seminal fluid (seminal plasma proteins) and semen quality (specifically sperm morphology (percent morphologically normal sperm)) were collected and stored for future evaluation.

Finally, other traits to be measured in the project included a comprehensive range of traditional measures of production (live weight, fatness, eye muscle area (EMA) and hip height).

Table I in Appendix III provides a detailed description of traits measured on tropical breed bulls and their dams.

3.2.2.2 Procedures for measurements of traits.

Live weight: Birth weight (LWT0) was recorded within 48hrs of parturition. Unfasted live weight was recorded using electronic weigh scales on the morning of the data collection date. Live weights were recorded at approximately 6, 9, 12, 15, 18, 21 and 24 months of age and the data were corrected for birth date.

Body Condition Score (BCS). A five-point scale with 1/3rd score increments was

[37]Entwistle, Fordyce (2003) *Australian Association of Cattle Veterinarians* eoaacv@bigpond.net.au) ISBN 0-9585654-4-9.
[38]Fordyce et al. (2006) *Theriogenology* **66,** 1140-1148.

adapted from a scale[39] cited from an earlier study of a body condition scoring system[40] used to describe body reserves of fat and muscling. This five-point scale included - 1 (poor), 2 (backward), 3 (moderate), 4 (prime) and 5 (fat).

Hip Height. Hip height (cm) was measured as the vertical distance from a fixed point to the top of the highest sacral vertebrae subtracted from the vertical distance from the fixed point to the ground at 15 months of age[39].

Rump Fat (P8). Rump fat (mm) was recorded as the real-time ultrasound-scanned subcutaneous fat depth at the P8 site (after "position 8" from the original research to define the optimum site for carcass fat measurement) on the rump (at the intersection of a line parallel to the spine from the *tuber ischium* and a line perpendicular to it from the spinous process of the third sacral vertebra[39 41].

Rib Fat. Rib fat (mm) was recorded as the real-time ultrasound-scanned subcutaneous fat depth between the 12th and 13th ribs[39].

Eye muscle Area (EMA). Eye muscle area (cm^2) was recorded as the real-time ultrasound-scanned cross-sectional area of the eye muscle (*M. longissimus thoracis et lumborum*) between the 12th and 13th ribs[39 41].

3.2.2.3 Adaptation phenotypes

Flight Time. Flight time was an electronically recorded time taken for an animal to cover a distance of approximately 2 metres after exiting a weighing crush[42]. Flight times were recorded twice at weaning (FT6a and FT6b) at ~ 7 days apart[43] and at 12, 18 and 24 months of age.

3.2.2.4 Blood hormonal phenotypes

GnRH-stimulated LH. Previous research has identified associations between GnRH-stimulated LH[13,14,15] and testosterone[14] and aspects of reproductive performance in post-pubertal tropically adapted genotypes managed under semi-extensive conditions; and between LH and age of puberty in pre-pubertal *Bos taurus* bulls[17,18,22] and as a useful early-in-life predictor of fertility[20] under intensive management conditions.

As a result, pilot studies were conducted by Jessica Crisp and the 'Male indicator traits to improve female reproductive performance' Project Team on industry Brahman and Tropical Composite pre-pubertal bull calves to develop a field protocol for a two-sample GnRH-stimulated LH response test for these tropically adapted beef bull calves[44]. A second objective was to determine an optimum dose rate of GnRH treatment and efficient sampling techniques to capture the LH response[44].

[39]Upton et al. (1999) *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **13**, 341-411.
[40]Lowman et al. (1976) *Bulletin No 6*, The East Scotland College of Agriculture, Animal Production, Advisory and Development Department, Edinburgh.
[41]Upton et al. (2001) *Australian Journal of Experimental Agriculture* **41**, 943-952.
[42]Burrow et al. (1988) *Australian Society of Animal Production*, **17**, 154-157.
[43]Burrow, Corbet (2000) *Australian Journal of Agricultural Research* **51**, 155-162.
[44]Crisp et al. (2012a, in preparation) *Theriogenology*.

Plate 1. Collecting blood plasma to determine LH response to a GnRH challenge at 3-4 months of age.

Brahman (n = 32) bull calves, 12 to 24 weeks of age were randomly selected at "Belah Valley" Station, Marlborough, in central Queensland (latitude 22º 42'S; longitude 149º 55'E) to determine this optimum dose rate. Calves were allocated to four GnRH treatment groups of 0.005 µg/kg, 0.05 µg/kg, 0.5 µg/kg, 5 µg/kg. Basal blood samples (lithium heparin BD Vacutainer® (Becton, Dickinson and Company)) were collected at Time 0 and calves were treated immediately post-sampling with intra-muscular GnRH (Fertagyl™, Intervet Australia Pty Limited). Further blood samples were then collected as close as possible to 32, 64, 96, 128 and 160 minutes post-treatment. Similarly, Tropical Composite (n = 28) bull calves, 12 to 24 weeks of age, were randomly selected at "Mt Eugene" Station, Jambin, in central Queensland (latitude 24º 08'S; longitude 150º 27'E). As with the Brahman calves, Tropical Composite calves were allocated to the four GnRH treatment groups of 0.005 µg/kg, 0.05 µg/kg, 0.5 µg/kg, 5 µg/kg, respectively. Basal blood samples were collected at Time 0 and calves were treated immediately post-sampling with intramuscular GnRH. Further blood samples were then collected as close as possible to 60, 120, 180 and 240 minutes post-treatment. Plasma samples were assayed for concentrations of LH. Plasma LH concentrations in all samples were measured by a double-antibody radioimmunoassay (RIA) procedure[45] that was modified by two scientific teams[46],[47] and by Ms. M. Blackberry, University of Western Australia.

Once the optimum dose rate of GnRH (Fertagyl™, Intervet Australia Pty Limited) was determined, the injection, collection and assay protocol discussed above was used to establish GnRH-stimulated LH levels in 3-4 month old Beef CRC Brahman and Tropical Composite bull calves.

Inhibin. Inhibin has been reported to be exclusively produced by Sertoli cells in the

[45]Martin et al. (1980) *Animal Reproduction Science* **3**, 125-32.
[46]Hotzel et al. (1998) *Journal of Reproduction and Fertility* **113**, 217-30.
[47]Hawken et al. (2009) *Biology of Reproduction* **80**, 1146-51.

testes[48] [49] [50]. Inhibin, which has also been linked to the regulation of spermatogenesis[50], increases fertility-

Plate 2. Collecting blood sera to determine Inhibin levels at 3-4 months of age.

associated characteristics before puberty[51]; has no antagonisms between it, FSH, LH and testosterone during pre-pubertal and post-pubertal stages of testicular development and function[52]; and its pre-pubertal serum level is directly related to scrotal circumference and sperm production in mature bulls[49].

At GnRH-stimulated LH Time 0, a basal whole blood sample (minimum 5mL) was collected from 3-4 month old calves at branding into 10mL Serum BD Vacutainer® tubes (Becton, Dickinson and Company). These sera were assayed for concentrations of inhibin by Monash University using established protocols[50].

Insulin-like Growth Factor-1 (IGF-1). Serum concentration of IGF-1 in pre-pubertal *Bos taurus* bulls has been reported to be positively correlated with adult scrotal circumference and sperm motility and genetically correlated with the age at first calf of female progeny and calving rate[21]. IGF-1 was also reported to be the best genetic predictor of age at first *corpus luteum (*age at puberty) in Brahman and Tropical Composite heifers in northern Australia[27]. Further, as blood serum IGF-1 appeared to be a promising predictor of fertility in *Bos taurus cattle*, it was evaluated in Brahman and Tropical Composite bull calves at weaning[4].

At weaning (~6 months of age), whole blood was collected onto bloodspot collection cards supplied by PrimeGRO™ to determine blood IGF-1 levels. IGF-1 was assayed

[48]Kaneko et al. (2001) *Biology of Reproduction* **65**, 209–215.
[49]Sharpe et al. (2003) *Reproduction* **125**, 769–784.
[50] Phillips (2005) *Domestic Animal Endocrinology* **28**, 1-16.
[51]Wheaton, Godfrey (2003) *Theriogenology* **60**, 933–941.
[52]Matsuzaki et al. (2000) *Journal of Reproduction and Development* **46**, 245–248.

using a commercially available (Rivalea (Australia) Pty. Ltd.) enzyme-linked immunosorbent assay[53].

Plate 3. Blood collected onto bloodspot collection cards supplied by PrimeGRO™ to determine blood IGF-I levels.

3.2.2.5 11β-Hydroxysteroid dehydrogenase, blood cortisol and corticosterone.

The 11β-hydroxysteroid dehydrogenase (11β-HSD1) enzyme catalyses the reversible inactivation of cortisol and corticosterone to their inert 11-ketosteroid metabolites, cortisone and 11-dehydroxycorticosterone[54]. Reduced expression or activity of 11βHSD in testes Leydig cells allows physiological concentrations of glucocorticoids to inhibit testosterone biosynthesis, resulting in a disturbance of spermatogenesis[54]. 11β-HSD1 has also been detected in the seminal plasma of dairy bulls (A. E. Michael, pers. comm.[4]).

Variation in the expression 11β-HSD1 in the testes of tropically adapted bulls may explain some of the observed variation in PNS between bulls, related to differences in susceptibility to episodes of testicular degeneration[55]. This study explored the levels of cortisol and cortisone in both the blood plasma and seminal plasma of tropically adapted Brahman and Tropical Composite beef bulls at 24 months of age. A blood sample (lithium heparin BD Vacutainer® (Becton, Dickinson and Company)) was collected directly before semen collection. Semen samples were collected, evaluated and stored in liquid nitrogen[25]. A blood plasma and seminal plasma sample for each bull was assayed to determine the concentrations of both cortisol and cortisone (the ratio of these provides an indication of the levels of the enzyme 11β-HSD which converts the biologically active cortisol to the inactive cortisone) using radioimmunoassay. The relationships between 11β-HSD1 and cortisol or cortisone levels and reproductive traits are currently being investigated by Prof. Michael McGowan, School of Veterinary Science, The University of Queensland, St. Lucia,

[53]Moore (2005) *Australian Journal of Agricultural Research* **56**, 211-218.
[54]Michael et al. (2003) *Reproduction* **126**, 425–441.
[55] Crisp et al. (2012e, in preparation). *Animal Reproduction Science*.

Qld, and other 'Male indicator traits to improve female reproductive performance' Project Team Members, to determine if they are a useful predictor or fertility. These results should be available by late May, 2014.

Plate 4. Extraction of plasma for determination of the concentrations of both cortisol and cortisone (the ratio of these provides an indication of the levels of the enzyme 11β-HSD).

3.2.2.6 Seminal Plasma Proteins

It is commonly accepted that seminal plasma proteins play a vital role in the physiology and development of sperm along the reproductive tract and also their fertilising capacity[56] [57] [58] [59] [60]. The hypothesis was that they could be early-in-life indicators of male and female reproductive performance. As reported earlier, this research activity comprised one aspect of Jessica Crisp's PhD and was funded through an MLA funded scholarship, an MLA funded Project (B.NBP.0507) and with additional top-up funds from the Beef CRC. This research activity has received supervision and assistance from Professor Arlindo Moura, Department of Animal Science, The Federal University of Ceará, Brazil. The results from this study and additional research aspects of this activity are currently being analysed and a publication is in preparation (December, 2013).

[56] Killian et al. (1993) *Biology of Reproduction* **49**, 1202–1207.
[57] Cancel et al. (1997) *Biology of Reproduction* **57**, 1293–1301.
[58] Brandon et al. (1999) *Theriogenology* **52**, 863–873.
[59] Roudebush et al. (2001) *Theriogenology* **55**, 1633–1638.
[60] Brackett et al. (2004) *Reproduction, Fertility and Development* **16**, 265.

At each BBSE, subsamples of each ejaculate were collected then deep frozen, initially into liquid nitrogen and then transferred to a -80 freezer. The initial investigation concentrated on 176 data sets from the 24 month BBSE of the 2005 Brahman bulls. PNS were grouped into deciles, and then 75 seminal plasma samples were randomly selected equally across deciles. Two dimensional electrophoresis was used to produce maps of the proteins present in the seminal plasma and the software PDQuest was used to quantify the expression (or amount) of each protein in 56 high quality maps. The identification of specific proteins was done by mass spectrophotometry procedures developed in collaboration with Professor Arlindo Moura. A multi-level statistical modelling procedure was used to analyse the results.

3.2.2.7 Conformation traits

Leg structure was based on a score of leg angularity on a scale of 1-9, with 9 for normal and 1 for an animal that could hardly walk because of incorrect angularity, either too straight or too bent[61]. A letter was also recorded with the leg structure score code to assist in explaining any problem associated with leg structure, i.e., pastern (P), straight hocks (T), sickle hocks (S), bowed legs (B), cow hocks (H) or stringhalt (C).

A score for feet structure was based on a scale of 1-9 with 9 being a perfect foot while 1 represents a foot that almost renders the animal a cripple[61]. A letter was also recorded with the feet structure score code to assist in explaining any problem associated with length of the toes (L), excessive curvature of the claws (C) or feet that were very low in the heel (H).

The tightness and pendulousness of the sheath was scored and ranged from 1 being extremely large to 9 being very small[61]. The scores included - 9 (tight), 7-8 (small), 5-6 (moderate), 3-4 (large) and 1-2 (very large).

An estimate of the length (mm) of preputial mucosa exposed when relaxed was scored[62].

An erection or otherwise of the penis was recorded if it occurred or did not occur during the electroejaculation procedure. That is, whether the bull had an erection (y), just a penile protrusion (p) or no appearance (n) of the penis[62]. It was noted that an erection was an individual bull response to the electroejaculation procedure. The penis structure/anatomy was reported as visually normal or abnormal.

Scrotal traits were recorded by experienced scorers trained and supervised by an ACV Accredited Examiner for a BBSE. Scrotal circumference of the testicles measured at the widest diameter is still the best method of assessing testicular development[63]. Scrotal circumference (SC) was measured with a standard metal tape[62 37]. Testicular tone was recorded as a score of the firmness and resilience of the testicles. This score ranged from 1-5 (1 = very soft, 3-4 ideal, 5 very hard[62], based on an ACV classification[37].

3.2.2.8 Semen collection traits

[61]Anon (1994) *Final Report UNE 30, MLA*, North Sydney, NSW 2059.
[62]Holroyd et al. (2002b) Chapter 3, pp. 3.1 - 3.19, *AACV Conference Proceedings*, AACV. Indooroopilly, Qld 4068. ISBN 0 9585654 3 0.
[63]Barth AD (2000) *The Western Canadian Association of Bovine Practitioners*, pp. 1–75.

Crush side collection and evaluation

Semen was collected with a CGS Electrojector (N2794, CGS Products Pty Ltd, Trafalgar, Victoria, Australia). Collection of an ejaculate was only attempted if scrotal circumference was ≥20 cm. If an animal did not produce an ejaculate using an electroejaculator, rectal massage was applied to the ampullae to determine if an ejaculate could be collected[37]. If an animal lay down in the crush during the electroejaculation procedure, an attempt was made to get the animal to its feet to continue the procedure and if this was not successful the animal was released from the crush. If an ejaculate could not be collected from an animal it was recorded as a missing value for the BBSE procedure.

Plate 5. Measurement of scrotal circumference with ACV Accredited metal tape.

Crush-side semen traits were based on the standards prescribed by the ACV[37] and scored by experienced scorers trained and supervised by an ACV Accredited Examiner for BBSE. These traits included -

(i) Volume (mL) of a fresh ejaculate at bull body temperature recorded crush side immediately after semen collection and used for sampling. The objective was to collect a minimum of 3mL of representative ejaculate.

(ii) Colour of a fresh ejaculate recorded crush side immediately after semen collection and scored on a scale of 1-5 with 1=pale, 2=cloudy to milky, 3=milky, 4=creamy, 5=yellow.

(iii) Density of the ejaculate recorded crush side immediately after semen collection on a scale of 1-5 with 1=dilute or clear to cloudy, 2=cloudy to milky, 3=milky, 4=creamy, 5=thick creamy or dense.

(iv) Mass activity (or wave motion) of a fresh ejaculate at bull body temperature recorded crush side immediately after semen collection. Mass activity was conducted using a Prism Optical, PRO 2300 Binocular Phase Contrast Microscope with an LEC warm stage. This activity was scored at X40 on a scale of 1-5 with 1 being no swirl, 2-3 slow distinct swirl, 4 moderate swirl and 5 swirl is in continuous dark waves.

(v) Motility was recorded crush side immediately after semen collection as the percentage of sperm viewed at X400 that were progressively motile by their own propulsion.

Plate 6. Electroejaculation of bull and collection of crush side semen sample.

Plate 7. Crush side evaluation of ejaculate – Mass Activity and Percent Motile.

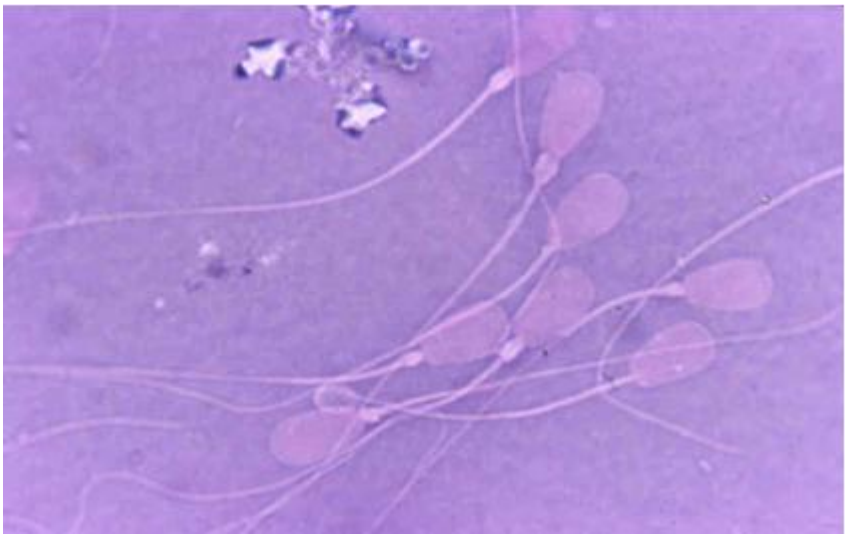

Plate 8. Crush side evaluation of ejaculate – Mass Activity and Percent Motile in a forward direction.

Laboratory evaluation of sperm morphology traits

Immediately after each crush side evaluation of an ejaculate, a number of 50ul aliquots of ejaculate, dependent on the quality of/concentration of sperm/cells in the ejaculate, were taken with a micropipette and placed into 2.95ml of Phosphate Buffered Saline (PBS) for sperm morphology assessment (maximum of 5 drops).

The sperm morphology assessment of each semen sample collected on every bull at 12, 18 and 24 months of age during this study was conducted by the same experienced technician who is an ACV Accredited sperm morphologist (research) and was responsible for assessing normal and abnormal sperm on each sample. Morphological assessment involved counting 100 cells systematically across a slide and noting the number of various abnormalities. Abnormalities were grouped into categories including head, midpiece, tail and droplets. A count of the normal cells allowed PNS to be calculated.

In a study conducted with tropical genotype bulls managed under extensive grazing conditions and in multiple-sire mated herds in northern Australia, PNS and the spermiogram were shown to be the best practical predictors of bull fertility[23,64]. As a consequence, based on earlier reviews, there was further investigation of PNS in this study to determine its relationship with female reproductive performance[35,36]. As the measurements on the bulls in this study were to be finalised at 24 months of age, it was not logistically possible to naturally mate and evaluate the calf-output of these bulls. While some bulls were used by the collaborators, the majority of these bulls were slaughtered after 24 months of age. Therefore, this study assessed the predictive value of PNS at 24 months of age based on the results of the MLA funded Bull Power Project (NAP3.117)[23] (see Plate 10)[23,25].

[64] Fitzpatrick et al. (2002) *Animal Reproduction Science* **71**, 39–49.

Plate 9. Morphological assessment of sperm involves counting 100 cells systematically across a slide and noting the number of various abnormalities.

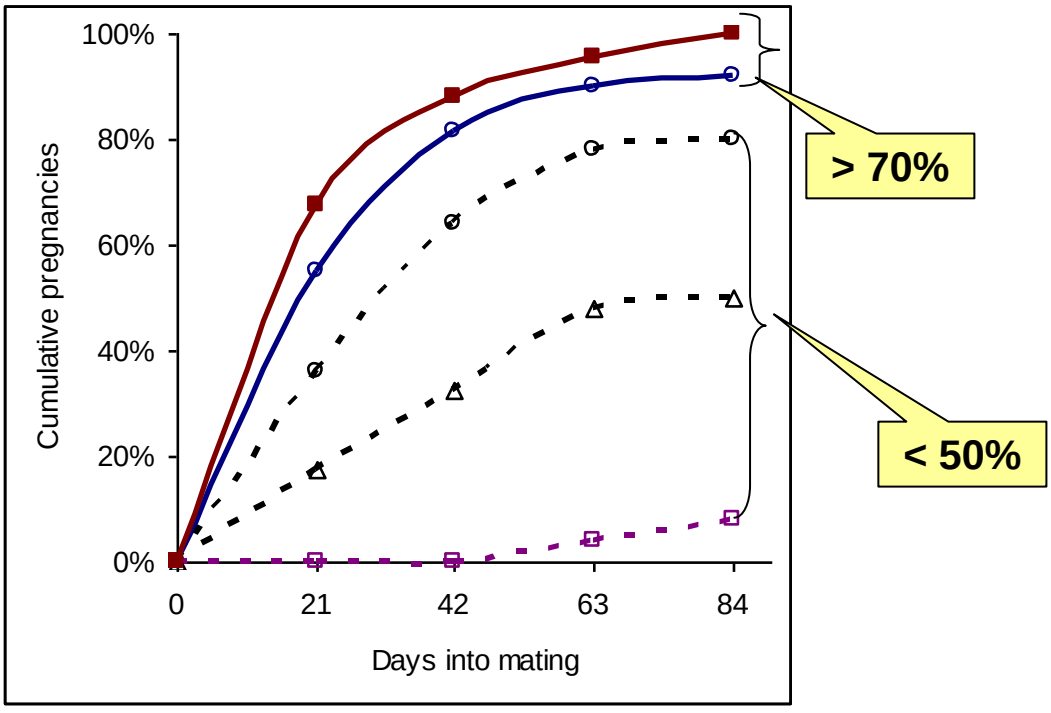

Plate 10. Bulls with >70% Normal Sperm compared to bulls with <50% Normal Sperm produce more and earlier pregnancies[23].

3.3 Statistical analyses

3.3.1 Phenotypic analyses

3.3.1.2 Systematic evaluation of the effects of environmental and management factors on bull phenotypes

Initial data editing excluded animals affected by illness or injury. Additionally, bulls with abnormal testicular development, particularly in the form of gross hypoplasia or cryptorchidism, were culled from the project and their records excluded from the data analyses. Deaths due to disease, accidental injury or unknown reasons also occurred during the course of the experimentation. Table II in Appendix III summarises the numbers of bulls exiting the project due to death or culling within genotype and age at exit. The total attrition of young bulls due to death and culling from weaning to 24 months of age amounted to approximately four percent.

Bull phenotypes representing growth, carcass, adaptation, hormonal, conformation, scrotal measurements, semen collection and quality and sperm morphology were evaluated in this study. The analyses models followed published procedures[24]. In summary, a univariate mixed model analysis of variance was performed using ASReml-R (version 3.0) for each genotype separately (Brahman or Tropical Composite) to identify significant main effects (environment and management) affecting key bull phenotypes. The fixed effects in the mixed model included - pre-weaning location, birth year, post-weaning location, dam origin, dam age, dam month of birth, dam paddock, dam previous lactation status and calf month of birth. The age of calf was fitted as a covariate and nested in calf month of birth. For Tropical Composites, additional terms of sire breed and dam breed groups were fitted in the model to account for heterosis effects. For all models, the random effects included sire and residual. Significant first order interactions were accounted for by concatenation of the fixed terms. The analysis was also conducted for each trait in a combined dataset. The breed difference among Brahman, Tropical Composite and Crossbred genotypes and their interactions with all main effects were examined in the combined data analyses. As the main effects were not factorial designs, the analyses were performed with all the main effects being nested within individual breed. For each variate, the parameters associated with the model were then produced for all fixed effects, including ANOVA significance values (P value), estimated least square (LS) means (LS mean) and LS effects.

3.3.1.3 Linear and non-linear phenotypic predictions for percent normal sperm at 24 months

Traits measured at weaning, 12, 18 and 24 months of age were examined for their phenotypic associations with the PNS at 24 months of age (PNS24) to see if any of these traits could be used as phenotypic predictors for PNS24. Four statistical models in R Program (version 2.13), namely linear mixed model (LMM), generalised linear mixed model (GLMM), non-parametric generalised additive models (GAM) and classification and a regression tree based model (TREE) were applied to evaluate the relationships at each time point, as well as all time points simultaneously in Brahman and Tropical Composite populations, respectively. PNS24 was analysed either as a continuous response variable with LMM, GAM and TREE or a binary variable using 70% as a threshold value with GLMM (i.e., “1” for PNS24 ≥70% or “0” for PNS24 <70%). The application of GAM and TREE aimed to capture significant non-linear relationships between PNS24 and multiple predictors, and to identify complex dependencies among predictor variables (i.e., interactions between important variables).

3.3.2 Genetic analyses

3.3.2.1 Fixed-effect modelling.

Significant fixed effects were identified separately for each genotype using linear mixed model procedures of SAS (SAS Institute, Cary, NC, USA) or GenStat (13th Edition). Models included the fixed effects of year (2004 to 2010), birth location (5 properties), birth month (September to January), post-weaning location (Brigalow or Belmont Research Stations), dam age (3 to 9 years) and previous lactation status (wet or dry), dam management group, their interactions and sire as a random effect. The effect of assay or sample group was included for blood hormone traits and age nested within birth month was included as a covariate for all traits. Ambient temperature was included as a covariate for rectal temperature records. Terms for sire group and dam group and their interaction were included to account for additive and possible non-additive breed and composite genotype effects in Tropical Composite and Crossbred genotypes. Non-significant terms were sequentially removed from the model to yield the final model for each variate.

3.3.2.2 Variance component estimation

Additive genetic variance and heritability for each trait was estimated in univariate analyses separately for each genotype using ASReml (v3.0). The animal models used included the final fixed effects identified above for each variate with an additional random common environmental effect of the dam when significant using log-likelihood ratio tests. Scrotal circumference at various ages was analysed with and without body weight as a covariate in the model. Genetic and phenotypic correlations between traits were estimated in a series of bivariate analyses with ASReml. For all analyses, a relationship matrix was derived from a pedigree of 17,020 animals spanning several generations. A total of 60 Brahman and 76 Tropical Composite sires were represented in the dataset with approximately 30 bull progeny produced per sire. Of these sires, 66 produced 20 or more sons with semen morphology records at 24 months of age.

3.4 Project staff

3.4.1 Research team by organisation

Table 4. Research team by organisation

DEEDI/DAFF	CSIRO Livestock Industries	Animal Genetics and Breeding Unit	University of Queensland
Richard Holroyd	Nicholas Corbet	David Johnston	Michael McGowan
John Bertram	Yutao Li		Jessica Crisp
Brian Burns (QAAFI)			Marina Fortes
Debra Corbet			
Vivienne Doogan			
Tim Grant			
Michael Sullivan			
Bronwyn Venus			
Jim Walkley (Beef CRC)			

3.4.2 Student involvement

3.4.2.1 Postgraduate Students

Marina Fortes (2007-2012) 'Genes and Genetic Markers Associated with Puberty in Beef Cattle' (PhD Thesis submitted and accepted – May 2012). MLA Funding for 'Markers and Genes influencing puberty in tropically adapted beef cattle' Project - $41,000. Marina has made a substantial contribution to the publication of project results relating to molecular genetics.

Jessica Crisp (nee Mayes) (2007-2013) 'Causes of variation in fertility of physically normal tropically adapted bulls' - MLA Postgraduate Scholarship/Study Award (1 April, 2007) of $100,000 plus $25,000 towards technical/operating. Jessica also received a Beef CRC top-up scholarship of $3,000 per year. Due to personal reasons, Jessica recently withdrew from her PhD program. Luteinising hormone (LH), inhibin and seminal plasma protein results generated from her experiments have been used in data analyses and have subsequently been used in other associated projects. Papers reporting the results of these experiments are currently being prepared.

3.4.2.2 Undergraduate Internships

Carley Bidstrup (2006) Genetic and non-genetic factors affecting reproductive performance in beef cattle in tropical central Queensland. Bachelor of Animal Production, The University of Queensland, Gatton, Internship Workplace Project, 10 September 2006.

Tracy Muller (2007) Determination of normal scrotal development in tropically adapted beef bulls. Honours Research Project, School of Animal Studies, The University of Queensland, Agricultural Science, Internship Workplace Project, 26 October, 2007.

Leah Sullivan (2007) Monitoring behaviour of bulls during breeding soundness evaluation. Industry Placement Project, University of Melbourne.

Zoe McInnes (2007) Does the age and previous calving history of the dam affect the growth rate of her offspring? Bachelor of Agricultural Science, Rural Management, The University of Queensland.

Alexander Thompson (2008) Longevity of breeding females in tropical beef herds - prevalence and reasons for mortalities and cullings. Bachelor of Agricultural Science, The University of Queendland, Internship Workplace Project, 31 October, 2008.

Cassie Duggan (2008) The contribution of bull and cow social and sexual behaviours to calf genotype in herds comprising two genotypes. Bachelor of Agricultural Science, The University of Queensland, Undergraduate Thesis, 27 October 2008.

3.4.2.3 Overseas Students

Bianca Fortuin (2006) Oestrus detection devices as an alternative to ultrasonography in tropical beef cattle. Internship Project, Bachelor of Science, Van Hall Instituut, Leeuwarden, The Netherlands.

Sophie Dupland (2006) Genetic and environmental effects on beef production in Australian tropical environments. Internship Project, Bachelor of Science, ENITA de Bordeaux, France.

Erik Schmidt (2008) Industry Placement, March-April 2008, Final year Veterinary Science, National University of Asunción, Paraguay.

Leonardo Canallas (2009) Industry Placement, February 2009, Master of Veterinary Science, Federal University of Rio Grande do Sul, Brazil.

4 Results and discussion

4.1 Phenotypic results and discussion

A large amount of data was collected during the course of this project. The tabulation of results from all data analyses can be found in Appendix III (Tables II to XIV) at the end of this report. The more significant and important findings are presented in the discussion that follows.

4.1.1 Systematic evaluation of the effects of environmental and management factors on bull traits

4.1.1.1 Age, live weight, scrotal circumference (cm), motility (%) and percent normal sperm by genotype

Due to circumstances, not all young bulls were measured for all traits. The 12-month BBSE was not conducted on the 2004 cohort as the Beef CRC had not officially commenced and the 2010 cohort had no BBSE or any of the post-weaning traits recorded as the Beef CRC was finalised before this information could be collected. At BBSE, only those bulls with SC of ≥20cm were electro-stimulated to collect an ejaculate sample. Previous experience deemed that young bulls with SC of less <20cm were sexually immature and were not able to provide an ejaculate with spermatozoa present. Table 5 summarises the number of young bulls presenting for BBSE, those that had a SC ≥ 20cm, and those that produced ejaculates with assessable sperm at each time point within each genotype.

Table 5. Numbers of young bulls by genotype, status and age at each Bull Breeding Soundness Evaluation (BBSE)

Genotype/ Status	Brahman			Tropical Composite		
	12 months	18 months	24 months	12 months	18 months	24 months
BBSE (N)	1340	1409	1403	1924	2081	2069
Stimulated[A] - SC≥20cm (N)	850	1374	1401	1863	2080	2068
Produced an Ejaculate (N)	807	1308	1390	1843	2064	2060
With assessable sperm[B] (N)	103	826	1234	970	1794	1912

[A] Bulls with SC of ≥20cm were electro-stimulated for ejaculate collection

[B] Bulls assessed for percent normal sperm (PNS); a PNS value could only be recorded if ≥100 spermatozoa were present in the fixed ejaculate subsample

The effects of genotype, birth location, month of birth and dam management group all accounted for significant proportions of variation in key bull reproductive traits. Predicted genotype means for key bull traits over time are presented in Table 6 and predicted means for key traits measured at 18 months of age by birth month for combined genotypes are presented in Table 7.

Table 6. Genotype means for key traits measured on bulls born and raised as contemporaries at Belmont Research Station

Time	Genotype*	Live weight (kg)	SC (cm)	Motility (%)	Normal (%)
12 months	Brahman	247	22	6	20
	Tropical Composite	290	27	43	48
18 months	Brahman	345	26	38	41
	Tropical Composite	370	31	54	59
24 months	Brahman	390	31	75	68
	Tropical Composite	414	33	73	66

*Numbers of animals were 547 Brahman and 527 Tropical Composite
SC = scrotal circumference (cm); Motility% = percent sperm motile in a forward direction evaluated crush side at X400 magnification; Normal% = percent morphologically normal sperm.

The results show an advantage to Tropical Composites in development of scrotal circumference and semen quality particularly at 12 and 18 months of age (Table 6). These differences could be exploited by choice of breeds and have implications for management decisions. However, the breed differences presented were derived from cattle running in moderately stressful environments of central Queensland compared to the harsher, more stressful environments to the North, and therefore may not apply in harsher conditions further north and west. In these harsher environments, the impact of higher parasitic and ambient temperatures and poorer nutritional factors on growth and subsequent sexual development should be considered when assessing likely genotype impacts on scrotal circumferences and semen quality. Scrotal circumference and semen quality threshold values may occur at older ages unless a targeted supplementation program is implemented to reduce of the impact of these environmental factors. Development of scrotal circumference and semen quality of young bulls was significantly affected by month of birth (Table 7 – combined genotypes) where development was delayed in later born calves. These results suggest that management to control the start of calving, its duration and the nutrition of pre-pubertal bulls can be used to improve growth and reproductive traits in young bulls.

Table 7. Model predicted means for key bull traits at 18 months of age by month of birth

Birth month	N*	Live weight 18 months (kg)	SC18 (cm)	Motility 18 (%)	Normal 18 %
September	579	391	30	59	69
October	1628	384	29	57	69
November	927	374	29	53	64
December	345	359	28	45	56
January	54	347	27	44	40
s.e.d.		*2.1*	*0.2*	*1.7*	*2.0*

*N = number of bulls in 2004 to 2009 birth-year cohorts; s.e.d. = standard error of the difference
Live weight 18 months = unfasted live weight recorded straight after placed in yards; SC18 = scrotal circumference at 18 months of age; Motility18 % = percent sperm motile in a forward direction at 18 months of age and evaluated crush side at X400 magnification; Normal 18 = percent morphologically normal at 18 months of age.

4.1.1.2 Hormonal traits

GNRH-stimulated LH

As reported earlier, this study sought to develop a functional field protocol for a double blood sample, GnRH-stimulated LH response test that could be used in further studies and also practically in northern Australian beef herds. The study also considered the feasibility of such a test under extensive northern Australian conditions and cattle husbandry practices, economical dose rates, and processing capabilities including animal group sizes, sampling times and cattle yard design. Experiments 1 and 2 were conducted on 12-24 week old Industry Tropical Composite and Brahman bull calves, located at 'Mount Eugene', Jambin, Qld. and 'Belah Valley', Marlborough, Qld., respectively.

The results for each genotype are presented in Tables 8 and 9.

Table 8. Mean LH responses of Tropical Composite bull calves (n = 28; 4 groups of 7 animals) to GnRH treatments[1] in Experiment 1 conducted at 'Mount Eugene', Jambin, Qld.

Time	Treatments[2]			
	0.005 µg/kg	0.05 µg/kg	0.5 µg/kg	5 µg/kg
0[1]	0.34^{de}	0.32^{e}	0.19^{e}	0.41^{d}
60	0.23^{e}	0.66^{de}	4.47^{b}	6.71^{a}
120	0.19^{e}	0.31^{e}	1.39^{d}	4.84^{b}
180	0.19^{e}	0.23^{e}	0.61^{de}	2.47^{c}
240	0.15^{e}	0.18^{e}	0.33^{e}	0.81^{de}

[1]GnRH was administered at Time 0 after the first basal blood sample was collected.
[2]Means with a common letter are not significantly different (P=0.05). Least Significant Difference (LSD) (P=0.05) = 0.0924 for comparing means within the same level of treatment and LSD (P=0.05) = 1.0511 for all other comparisons.

Table 9. Mean LH responses of Brahman bull calves (n = 32; 4 groups of 8 animals) to GnRH treatments[1] in Experiment 2 conducted at 'Belah Valley', Marlborough, Qld.

Time	Treatments			
	0.005 µg/kg	0.05 µg/kg	0.5 µg/kg	5 µg/kg
0	0.18[d]	0.19[d]	0.23[d]	0.26[d]
32	0.17[d]	0.30[d]	4.89[b]	7.43[a]
64	0.17[d]	0.21[d]	1.67[c]	7.38[a]
96	0.15[d]	0.15[d]	0.56[d]	5.25[b]
128	0.14[d]	0.13[d]	0.30[d]	2.26[c]
[2]Means	[2]Means	[2]Means	[2]Means	[2]Means

[1]GnRH was administered at Time 0 after the first basal blood sample was collected.
[2]Means with a common letter are not significantly different (P=0.05). LSD (P=0.05) = 0.883 for comparing means within the same level of treatment and LSD (P=0.05) = 1.109 for all other comparisons.

From the responses recorded to the GnRH challenge in the pilot studies, it was decided to re-evaluate the GnRH challenge tests to determine if the LH responses peaked at an earlier time post-GnRH injection.

Experiment 3 was conducted 12 months after the results of Experiments 1 and 2 were assessed. Tropical Composite (n = 27) and Brahman (n = 27) bull calves that were approximately 12 weeks of age were randomly selected from the Northern Australia Beef CRC Breeding Project experimental herd at Belmont Research Station near Rockhampton, in central Queensland (latitude 23º 13'S; longitude 150º 23'E). All Tropical Composite calves were processed on one day and the Brahman calves the following day. Calves were allocated to three GnRH treatment groups of 0 µg/kg (control), 0.5 µg/kg and 1.5 µg/kg, respectively. The average body weight of the Tropical Composite calves was 131 kg (range 88 to 178 kg) and the Brahman calves was 118kg (range 80 to 173 kg). A basal blood sample was taken at Time 0 and calves were treated immediately post-sampling with an intramuscular injection of GnRH. Further blood samples were then taken as close as possible to 20, 40, 70 and 100 min post-treatment. The results from this experiment are presented in Tables 10 and 11.

Table 10. Mean LH responses of Tropical Composite bull calves (n = 27; 3 groups of 9 animals) to GnRH treatments[1] in Experiment 3 conducted at Belmont Research Station, *via* Rockhampton, Qld.

Time	Treatments		
	0 µg/kg	0.5 µg/kg	1.5 µg/kg
0	0.63[d]	0.60[d]	0.45[d]
20	0.60[d]	10.45[b]	16.52[a]
40	0.35[d]	6.64[c]	9.57[b]
70	0.22[d]	2.46[d]	3.99[c]
100	0.32[d]	1.20[d]	1.93[d]

[1]GnRH was administered at Time 0 after the first basal blood sample was collected.
[2]Means with a common letter are not significantly different (P=0.05). LSD (P=0.05) = 2.40 for comparing means within the same level of treatment and LSD (P=0.05) = 3.06 for all other comparisons.

Table 11. Mean LH response of Brahman bull calves (n = 27; 3 groups of 9 animals) to GnRH treatments[1] in Experiment 3 conducted at Belmont Research Station, *via* Rockhampton, Qld.

Time	Treatments		
	0 µg/kg	0.5 µg/kg	1.5 µg/kg
0	0.27^{e}	0.60^{de}	0.47^{de}
20	0.20^{e}	5.32^{b}	7.81^{a}
40	0.16^{e}	3.05^{c}	4.84^{b}
70	0.15^{e}	1.18^{d}	1.78^{c}
100	0.15^{e}	0.57^{de}	0.79^{cde}

[1]GnRH was administered at Time 0 after the first basal blood sample was collected.
[2]Means with a common letter are not significantly different (P=0.05). LSD (P=0.05) = 1.28 for comparing means within the same level of treatment and LSD (P=0.05) = 1.59 for all other comparisons.

From these three experiments, it was concluded that a dose rate of 0.5 µg/kg of GnRH was sufficient to elicit a significant LH response when captured 20 minutes post-GnRH treatment and was recommended for use in further studies[44].

Seminal Plasma Proteins

A multi-level statistical modelling procedure demonstrated that 8 proteins and their interactions accounted for 88% of total phenotypic variation in PNS in the Brahman and Tropical Composite bulls evaluated in this project[65]. Several seminal plasma proteins have been previously shown to be significantly related to conception rate in Holstein bulls[56]. This is the first comprehensive proteome report of seminal plasma proteins in Australian tropically adapted beef cattle breeds. More information will become available on these seminal plasma proteins with the completion of the analysis of this data (published by December, 2013)

Hypothetically, it was envisaged that a seminal plasma test may be able to be conducted crush side, and even at an earlier age, and offer some advantages over a full morphological sperm test which needs to be evaluated at a laboratory. It may have also added to the accuracy of the PNS test. The project was terminated on advice form the project team in June 2010 due to analytical difficulties and the prohibitive cost of analyses. The modified mass spectrophotometric analysis of a further 150 selected samples is being conducted to validate relationships between PNS and the 8 proteins. Should this prove both successful and useful, further funding will be sought to test larger numbers of stored samples to estimate phenotypic and genetic correlations between these proteins and a suite of male and female reproductive traits.

11β-Hydroxysteroid dehydrogenase

To date, semen and plasma samples from 120 tropically adapted bulls are being assayed for cortisol and cortisone levels and we are awaiting laboratory confirmation of the completion of these assays. This data will then be combined with bull fertility data collected at BBSE collections to ascertain their value as predictors of male reproductive phenotypes. These findings are additional to the core study and have arisen out of the PhD study of Jessica Crisp. While the results are yet to be finalised, they are not central to the outcomes of this report.

[65] Crisp et al. (2012d, in preparation). Animal Reproduction Science.

4.1.2 Linear and non-linear phenotypic predictions for percent normal sperm at 24 months

This activity was an attempt to find phenotypic predictors of male fertility. PNS at 24 months of age in Brahman bulls was more sensitive to environmental factors than in Tropical Composite bulls. The environmental and management factors included cohort, dam origin, dam management group, post-weaning lactation status and calf month of birth. Together these factors explained about 10% of variation in PNS at 24 months of age, while, in Tropical Composites, cohort was the only factor influencing PNS at 24 months of age and it explained only 4% of variation.

The comparisons of four statistical models (LMM, GLMM, GAM and TREE) demonstrated complex relationships between PNS at 24 months of age, the range of candidate male indicator traits and the impact of model selection on the results. Both LMM and GLMM identified PNS 12 and PNS 18 and sperm motility at 18 months in Tropical Composite as being useful predictors of PNS at 24 months of age, but individually they only explained a small percent of the total variation of prediction equations. The significant predictors identified in Brahmans were SC12 and MOT24, SC18 and MOT24, MAS18 and MOT24 and MOT18 and MOT24, and PNS18 with PNS 24. However, despite extensive model examination of the relationships between PNS at 24 months of age and multiple predictors, none of the bull traits could predict PNS at 24 months of age with a high statistical power. The results suggest that phenotypes are not strong predictors of male reproductive performance. Individual bulls should continue to be assessed for their fertility on the basis of a BBSE which includes PNS at around 2 years of age.

4.2 Genetics results and discussion

A detailed tabulation of heritabilities and genetic correlations among all bull traits measured are presented in Table I., Appendix III. Genetic correlations between bull traits and components of cow reproductive performance are reported by David Johnston in the 'Early predictors of lifetime female reproductive performance' MLA Final Report, NBP.0363) and in a recently accepted paper in Animal Production Science, Johnston et al. (2013, accepted) (see full reference In Section 9 Bibliography)

4.2.1 Genetic parameter estimates for bull traits

Genetic parameters (heritabilities and genetic correlations) estimated for key traits measured on Brahman and Tropical Composite bulls in this project[66] are summarised in Tables 12 and 13. The heritability estimates for hormonal and semen quality traits (Table 12) have not been published previously for tropical beef breeds. Heritabilities of the traits recorded were generally moderate (semen quality traits) to high (hormonal traits) indicating that genetic change could, in most cases, be readily made by selection. Scrotal circumference and hormonal traits were among the most heritable and genotype differences were evident.

[66] Corbet et al. (2013) *Animal Production Science*, 53 (2):101-113.

Table 12. Heritabilities of key male reproductive traits

Category	Trait	Brahman	Tropical Composite
Hormones	IN4	0.74 (0.09)[1]	0.72 (0.10)
	LH4	0.31 (0.10)	0.48 (0.08)
	IGF6	0.44 (0.08)	0.36 (0.07)
Testes	SC12	0.65 (0.08)	0.46 (0.09)
	SC18	0.75 (0.09)	0.43 (0.09)
	SC24	0.75 (0.09)	0.47 (0.09)
Semen quality	Mass activity 18	0.24 (0.07)	0.13 (0.05)
	Motility % 18	0.15 (0.06)	0.15 (0.05)
	Normal % 18	0.25 (0.09)	0.20 (0.06)

IN4 = Inhibin at 4 months of age; LH4 = Luteinising hormone at 4 months of age; IGF6 = insulin-like growth factor at 6 months of age; SC12 = scrotal circumference at 12 months of age; SC18 = scrotal circumference at 18 months of age; SC24 = scrotal circumference at 24 months of age; Mass activity 18 – mass activity (score 1-5) of sperm measured crush side at X40 magnification; Motility% 18 = percent sperm motile in a forward direction at 18 months of age; and Normal% = percent morphologically normal at 18 months of age. [1]Standard Error values

Genetic correlations among some key bull traits are presented in Table 13. The magnitude of positive genetic correlations changed across time (from 12 to 24 months of age) and genotype for some traits and this may impact on recording protocols within breeds if these traits are to be included in genetic evaluation programs. Genetic correlations among the bull traits generally suggested no antagonisms between production-type traits and reproductive traits. This result indicates that selection for semen quality, for example, will not adversely affect economically important growth traits.

Table 13. Genetic correlations for Brahman (above diagonal) and Tropical Composite (below diagonal) for some key bull traits

Trait	IGF6	WT15	EMA15	SC18	Motility 18 %	Normal 18 %
IGF6	-	0.53 (0.11)	0.46 (0.13)	0.49 (0.10)	0.35 (0.19)	0.44 (0.20)
Weight 15	0.19 (0.09)	-	0.51 (0.12)	0.35 (0.11)	-0.06 (0.21)	0.05 (0.23)
EMA15	0.34 (0.10)	0.59 (0.07)	-	0.14 (0.13)	-0.01 (0.22)	0.16 (0.23)
SC18	0.28 (0.12)	0.66 (0.6)	0.25 (0.12)	-	0.79 (0.10)	0.50 (0.13)
Motility 18 %	0.21 (0.17)	0.04 (0.16)	-0.17 (0.16)	0.44 (0.15)	-	0.86 (0.13)
Normal 18 %	-0.01 (0.15)	-0.05 (0.14)	-0.04 (0.14)	0.21 (0.16)	0.80 (0.12)	-

IGF6 = insulin-like growth factor at 6 months of age; Weight 15 = weight at 15 months of age; EMA15 = eye muscle area (cm^2) at 15 months of age; SC18 = scrotal circumference at 18 months of age; Motility% 18 = percent sperm motile in a forward direction at 18 months of age; and Normal 18 % = percent morphologically normal sperm at 18 months of age.

As a rule of thumb, a genetic correlation between two traits of between 0.4 and 0.7 indicates that one trait moderately affects the other, is therefore a useful indicator of the correlated trait, and measurements for one trait can contribute to the estimation of the genetics for the second trait. Genetic correlations between two traits of greater than 0.7 indicate that one trait strongly affects the correlated trait, is therefore a

powerful indicator of the second correlated trait, and as a result only one trait needs to be measured. Therefore, although IGF6 is moderately genetically correlated with SC and semen quality in Brahmans (0.35 to 0.49; Table 13) and has a moderate heritability (0.44; Table 12), it would only be of significant value as a screening test at weaning if the correlations were high, eg >0.70.

Despite relatively weak to moderate phenotypic correlations (see Tables XI and XII in Appendix III), there were generally moderate to strong genetic associations of scrotal circumference and sperm motility with PNS at 18 months, particularly in Brahman bulls (0.50 and 0.86; Table 13). These genetic associations suggest that scrotal circumference and sperm motility traits are potential early-in-life selection criteria for genetic improvement of bull fertility.

4.2.2 Genetic correlations between male and female reproductive traits

The genetic relationships between male and female reproductive traits were reported by David Johnston in the Beef CRC 'Early predictors of lifetime female reproductive performance' MLA Final Report, NBP.363) and have just been accepted for publication[67] (Johnston *et al.* 2013, accepted) (see full reference In Section 9 Bibliography). For this report, those results have been summarised here for key male and female traits in Table 14.

The male traits, IGF-1, SC and measures of semen quality, were genetically related to female age of puberty confirming results from preliminary work[68]. Male traits, particularly semen quality traits, were also genetically linked to first post-partum anoestrus interval, a critical measure of the ability of the first-calf cow to re-breed. Genetic correlations between male traits and lifetime weaning rate were generally of low magnitude, accompanied by high standard errors and were variable between genotypes. Except for sperm motility traits such as Mass Activity and Motility in Brahmans, male traits were relatively poor predictors of lifetime weaning rate. Some male traits could be flagged as potential indirect selection criteria to improve reproduction especially if they are more heritable and easier to measure than the cow reproduction traits. Preputial eversion is not routinely recorded in bull soundness examinations but it was strongly correlated with life time annual weaning rate in Brahmans -0.71 (0.27) and may have some role in future data collection strategies. Further discussion and recommendations are made by David Johnston in the Beef CRC 'Early predictors of lifetime female reproductive performance' MLA Final Report, NBP.363) and in a recently accepted paper in Animal Production Science, Johnston et al. (2013, accepted) (see full reference In Section 9 Bibliography)[67].

[67] Johnston et al. (2013, accepted). *Animal Production Science.*
[68] Corbet et al. (2011) *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **19**, 55-58.

Table 14. Genetic correlations between key male (bull) and female reproduction traits

	Female Trait		
Bull Trait	**Age at Puberty**	**PPAI**	**AWR6**
Brahman			
IGF6	-0.56 (0.11)	-0.21 (0.15)	0.20 (0.19)
SC18	-0.27 (0.10)	-0.27 (0.13)	0.12 (0.17)
Mass18	-0.51 (0.17)	-0.27 (0.18)	0.54 (0.26)
Motility18	-0.49 (0.20)	-0.37 (0.22)	0.51 (0.28)
Normal% 18	-0.48 (0.21)	-0.52 (0.31)	-0.01 (0.31)
Tropical Composite			
IGF6	-0.43 (0.11)	-0.10 (0.18)	-0.02(0.17)
SC18	-0.17 (0.11)	0.13(0.16)	0.14 (0.16)
Mass18	-0.24 (0.20)	-0.68 (0.36)	0.21(0.25)
Motility%18	-0.38 (0.18)	-0.73 (0.35)	0.29(0.24)
Normal% 18	-0.24 (0.17)	-0.30 (0.25)	0.41(0.22)

[1]PPAI = First post-partum anoestrus interval; AWR6 = Average Weaning Rate (retained cows) (=Lifetime weaning rate of surviving cows at 6th mating); IGF6 = insulin-like growth factor at 6 months of age; SC18 = scrotal circumference at 18 months of age; Mass18 – mass activity (score 1-5) of sperm at 18 months of age measured crush side at X40 magnification; Motility% 18 = percent sperm motile in a forward direction at 18 months of age and measured crush side; and Normal% 18 = percent morphologically normal at 18 months of age.

4.3 Overall results and discussion

A comprehensive list of all the traits on which information has been collected is presented in Table 15. While some of the growth and carcass traits have moderate to high heritabilities, they are not well correlated with PNS or cow reproductive traits and are therefore of little use as selection criteria to improve herd reproduction in either breed.

Of the adaptive traits, flight time may have some limited value in selection for female reproduction but the genetic correlations were low. In any case, temperament is already a screening trait on most properties.

The one hormonal trait that appears to have modest value for inclusion in a selection index is IGF-1, and only for Brahmans, but it is doubtful whether collecting blood samples from 6 month old calves would be justified given its low correlation with life time annual weaning rate. .

The conformational trait of sheath score has a moderate to strong genetic correlation with reproduction traits and given the relative ease and cost of recording, its inclusion in a BBSE is recommended. It should also be used as part of a selection index, however, currently it is not routinely collected nor is it part of the BBSE for bulls. Therefore, a whole new training and extension push would be needed so it can be accurately measured and recorded.

Despite a low heritability, semen motility is highly correlated with PNS and cow reproduction and should prove useful in a selection index. PNS itself was moderately heritable, is objectively assessed in accredited laboratories and is currently regarded as a useful pre-sale requirement for all stud/seedstock bulls. PNS and sperm motility offer exciting possibilities as selection tools for improved reproductive performance, especially in Brahmans, as they are moderately to highly correlated with female reproduction in that breed. The routine collection of these semen traits is strongly recommended in young bulls but the process for new traits (Table 16) will require all

bulls in birth-year groups to be collected, assessed and records uploaded onto the BREEDPLAN database – not just the records of sale bulls that pass the BBSE. Further, for an EBV to be developed for a trait, about 5,000 phenotypes per breed are required from industry herds for parameter estimation. Therefore, the key requirement is to obtain industry data (Hans Graser, pers. comm.).

There are obvious implications from the results above and other recent findings from Beef CRCIII projects for all producers and not just for seedstock producers that submit data to BREEDPLAN (Table 17). The primary goal should always be to select bulls from the most fertile breeders in the herd. The strategies for identifying these breeders are summarised as follows:-

(i) Do not to keep breeding females in the bull breeding unit that fail to produce (rear) a calf every year.
(ii) Certainly cull all cows that missed in two consecutive joinings (there may be exceptions for calf loss from misadventure/disease or predation).
(iii) Cull all heifers that do not conceive at the first joining if they were above their critical joining weight.
(iv) Purchase seedstock bulls that have some information on the fertility of the dams from which they were derived. Preferably, these bulls should have an EBV for days to calving (DTC) and at the minimum, a history of their female relatives' previous calving outcomes.
(v) Measure and select replacement herd bulls on scrotal circumference and semen traits such as motility, mass activity and PNS – select bulls with good temperament and good sheath score.
(vi) Overmate maiden heifers and select replacement heifers for the breeder herd on their ability to conceive as quickly as possible after the bull has been exposed to the herd - especially select heifers that reconceive within 3 months of calving.
(vii) In addition to fertility traits, if breeding for polled animals, ensure that all natural polled animals are clearly identified at the time of dehorning.

The uptake of these strategies will vary with individual circumstance and enterprise breeding objective (as predicted in Table 17.).

Table 15. Bull traits analysed and potential as alternative selection criteria to improve herd reproduction

Category	Trait	Time Measured (months)	Heritability[1] BRAH	Heritability[1] TCOMP	Genetic correlation with PNS24 BRAH	Genetic correlation with PNS24 TCOMP	Genetic correlation with Cow Reproduction BRAH	Genetic correlation with Cow Reproduction TCOMP	Trait Usefulness	Comments
Growth	Weight	15	Mod	High	Zero	Zero	Low	Zero	No	
	Condition score	15	Mod	Mod	Mod	Zero	Zero	Low	Possible	BRAH semen quality only
	Hip height	15	Mod	High	Zero	Low	Zero	Low	No	
	Rump fat	15	Mod	Low	Low	Zero	Low	Low	No	
	Rib fat	15	Low	Low	N/R	N/R	N/R	N/R		No residual
	EMA	15	Mod	High	Low	Zero	Low	Low	No	
Adaptation	Flight time	6	Mod	Mod	Zero	Zero	Low	Low	Possible	
	Coat score	6	Mod	Mod	Mod	Zero	Low	Zero	No	BRAH maybe
	Rectal temp	12	Mod	Low	Zero	Low	Low	Zero	No	High Standard Error
Hormonal	Inhibin	4	High	High	Low	Low	Low	Low	No	Inconsistent associations
	Stimulated LH	4	Mod	Mod	Low	Zero	Mod	Mod	Possible	BRAH only
	Insulin-like Growth Factor-1	6	Mod	Mod	Mod	Zero	Mod	Low	Yes	BRAH only
Conformation	Leg structure	18	Zero	Mod	Low	Mod	N/A	N/A		Low residual in BRAH
	Foot structure	18	Low	Mod	Low	Zero	N/A	N/A		Low residual in BRAH
	Sheath score	18	Mod	Mod	Low	Low	Mod	Mod	Yes	
	Prepuce eversion	18	Mod	Mod	Low	Zero	Mod	Mod	Possible	
Scrotal	Scrotal circumference	18	High	Mod	Mod	Mod	Low	Low	Yes	Heritable, easy to measure
	Testicular tone	18	Low	Low	Low	Low	N/R	N/R		No residual
Semen collection	Volume	18	Low	Low	Low	Low	N/A	N/A		Full volume not collected
	Density	18	Low	Low	Mod	Mod	N/A	N/A		Similar score to Mass Activity
	Mass Activity	18	Mod	Low	Mod	Mod	Mod	Mod	Yes	
	Motility	18	Low	Low	High	Mod	High	Mod	Yes	
	Normal sperm (%)	18	Mod	Mod	High	High	Mod	Low	Yes	

[1]Heritability levels (range 0 to 1) – Low (0 to <0.2); Moderate (0.2 to 0.4); and High (>0.4).
[2]Correlation levels (range -1 to +1) – No significant genetic correlation (-0.15 to +0.15); Low (-0.15 to -0.4 and +0.15 to +0.4); Moderate (-0.4 to -0.7 and +0.4 to +0.7); and High (-0.7 to -1.0 and +0.7 to +1.0). BRAH = Brahman; TCOMP = Tropical Composite; [2]Correlations highlighted in red denote negative or unfavourable genetic relationships.

Table 16. Current status of bull selection criteria

Trait	Current Status	Steps for Implementation
Scrotal circumference	Existing	• EBVs currently available on bulls registered on BREEDPLAN • Measurement at 12 to 18 months is optimal • Need to measure all young bulls in birth-year cohort to capture accurate genetic merit • One-off measurements of SC on sale bulls not meaningful
IGF-1 Sheath / prepuce Mass Activity Motility Normal%	New	• Accumulate a few thousand records within breed; some breeds (e.g. CRC breeds and Santa Gertrudis, Droughtmaster) may already have good data recorded for BBSE traits – need a system to capture/transfer BBSE information electronically. • Upload records to AGBU for testing of genetic parameters • Initiate trial BREEDPLAN runs

Table 17. Scenarios for adoption of genetic technologies by industry using different levels of performance recording (after McCosker 2011[69])

	Buyers	Bull Breeders	Seedstock Producers
Recording	No recording	Record at least scrotal circumference (SC) and days to calving Records submitted to BREEDPLAN	DNA samples at birth; pedigree; genomic prediction; polled test Birth weight; birth date; flight time Bull Breeding Soundness Evaluation (BBSE) including SC at 12 months in Brahmans and semen quality in Tropical composites. Controlled mating; pregnancy test at weaning
Bull selection	Establish breeding objective and buy bulls with estimated Breeding Values (EBVs) and indexes to suit	Top home bulls selected; not from cows that miss at first and second joinings Bulls also purchased from studs with efficient breeding program Cows culled if missed in two consecutive years	Bulls kept from high accuracy sires and dams (that calve each year) with EBVs suited to clear breeding objective Cows and heifers that miss are culled Scan heifers at joining for CL, muscle and fat
Benefits/consequences	Selection solely for weight traits will not improve fertility Ensure bulls have EBVs for reproduction traits Genetic trend stable or slightly positive	Able to sell herd bulls Genetic trend for reproduction is positive	Flexible turn-off strategies Sell top-price stud bulls Strong reproduction genetics; lactating-cow weaning rates improved by 15% in 10 years. Dependent commercial herds achieve same rate of genetic gain as bull source – lag period.

[69] McCosker T (2011) The poor financial performance of the northern beef industry – its causes and cures. In '*Proceedings of the Northern Beef Research Update Conference*, Darwin, 3–4 August 2011'. pp. 5–10.Edited by the R. G Holroyd., Published by the Northern Beef Research Update Committee.

5 Success in achieving objectives

5.1 Key outcomes and outputs

Objective 1. Identify early-in- life predictors of fertility both phenotypically (bull fertility as reflected by improved calf output) and genetically (the fertility of bull's female and male progeny, such as age at puberty).

Objective 2. Improve lifetime reproductive performance of females.

The project evaluated a large number of candidate, early-in-life predictors of an individual bull's phenotypic fertility (as indicated by PNS at 24 months) but none were of value. Individual bulls should continue to be assessed for their fertility on the basis of a BBSE which includes PNS at around 2 years of age.

The same candidate predictors were assessed for their value in assessing the relative genetic merit of bulls with respect to the fertility of their progeny. The latter was based on PNS at 24 months for males, and for females, age at puberty, first lactation anoestrous period, and lifetime reproductive rate of related females.

For males, the value of scrotal size as an indicator of genetic merit for male fertility was confirmed but its value for female fertility was relatively low for both genotypes. IGF-1 at 6 months of age showed some value for predicting genetic merit of both males and females in Brahmans but the genetic correlation was not high enough to warrant its use as an early-in-life predictor of fertility.

The semen quality traits, that is, mass activity, motility and PNS, show promise as predictors of components of female reproduction such as age at puberty and PPAI. Mass activity and motility were also strongly correlated genetically with lifetime reproductive rate of Brahman cows.

The relative merit of these candidate traits requires further work as they vary somewhat, within and across genotypes, in heritability and the strength of their genetic relationships with components of female reproduction. The reliability and ease of measurement also need to be considered. For Brahmans, PNS appears to be the most useful of the semen traits, especially given it is already assessed as part of the BBSE.

In combination with the related northern Beef CRC R&D (Gene Discovery for Post-Partum Reconception and Age at Puberty, B.NBP.0364; Early predictors of lifetime female reproductive performance, B.NBP.0363), this project has found that components of male and female reproduction in tropical breeds are under significant genetic control. This confirms earlier studies on improving reproductive rate in tropical cattle (eg, Hetzel et al. 1989) and re-enforces the urgency for tropical bull breeders to be collecting the key data required for genetic improvement such as the data required for EBVs on scrotal size and days to calving. There are also major differences between genotypes in reproductive performance which can be exploited by industry in appropriate environments.

There is major scope to improve the fertility of Brahmans, and semen traits at 18 and 24 months of age and scrotal circumference at 12 months of age have emerged as very useful traits for this breed, given the moderate heritabilities and moderate to strong genetic correlations with components of female reproduction. Motility traits

such as mass activity and motility are more strongly related to lifetime reproductive rate (albeit with a large standard error) but lifetime weaning rate has a low heritability.

5.2 Publications

To date 52 publications have been presented, another 20 have been submitted and many other papers are still in preparation. See Appendix II for details of the 'list of publications and presentations/activities' generated from the project.

6 Impact on meat and livestock industry – Now and in five years time

6.1 Next steps

Holmes (2010) reported that an increase in weaning rates in north Australian breeding herds of five percentage points at all first, second and subsequent calvings could result in increases in gross margins of approximately $5M; $9M and $30M per annum (based on 4 million breeders), respectively[69]

Using the project's genetic parameter estimates, the prediction of the response to selection for semen traits (proposed new selection trait) alone indicates that genetic progress of 6% in the lifetime annual weaning rate (LAWR – (Mating ≤ 6) - Total number of calves weaned divided by number of matings) over 10 years can be achieved as presented by Dr. David Johnson at the Final Beef CRC Forum, 12-14 June 2012, Auditorium, Queensland Biosciences Precinct, University of Queensland, Carmody Road, St Lucia. In a recent publication, Barwick et al. (2013, accepted) reported that the estimated increase in LAWR in 10 years, for combinations without or with genomic measures, ranged from 8 to 12 calves weaned per 100 cows from selection of sires, and from 12 to 15 calves weaned per 100 cows from selection of sires and dams. In addition, Barwick et al. (2013, accepted) also reported corresponding reductions in lactation anoestrous interval (LAI - (days; mating 2 and days to cycling of lactating cows) of 60 to 103 days or 94 to 136 days, and those for age at puberty (AGECL; Age at first *corpus luteum,* days; 10–40 months) were 95 to 125 or 141 to 176 days, respectively. Further, Barwick et al. (2013, accepted) reported that percent normal sperm may be important to record for reducing LAI and scrotal size and serum insulin-like growth factor-I concentration in heifers at 18 months for reducing AGECL. Barwick et al. (2013, accepted) also reported that the use of a genomic EBV in combination with other measures added to genetic gains, especially at genomic EBV accuracies of 40%. Finally, Barwick et al. (2013, accepted) concluded that accuracies of genomic EBVs needed to approach 60% for the genomic EBV to be the most important contributor to gains in the combinations of measures studied. Therefore, it is expected that integration of semen traits into EBVs will accelerate the rate of improvement in LAWR above that achievable using currently available EBVs for scrotal size and days to calving.

Theoretically, it should be possible to achieve genetic improvement in reproductive rate from using selection based solely on one or more semen traits.

Therefore, this project and its associated activities [66] [68] [70] [71] has clearly demonstrated to industry –

[70] Burns and Corbet (2012) The Bayer and Bioniche International Beef Cattle Genetics Conference, Central Queensland University, Rockhampton, 6-7 May, 2012. pp. 10.

(i) The value in seedstock producers recording scrotal circumference in Brahmans at 12 months and some BBSE traits in tropical composites at 12 months for improving current herd reproductive performance; and
(ii) The potential to use male traits to improve bull fertility and for indirect selection to genetically improve herd reproductive performance (via daughters) in northern Australia.

Steps needed to deliver results to industry include –
(i) The accuracy of genetic correlation estimates between male and female reproductive traits from this project need to be enhanced by ongoing collection and recording of traits within these Beef CRC research breeds and other tropical breeds to determine which male traits will be the key measures of herd reproductive performance. Establish the utility of measuring these bull traits in seedstock herds for selection programs by -
(a) Assessing the difficulty of measuring these traits at the industry level; and
(b) Assessing the costs of data collection *versus* financial returns of using these traits;
(ii) Develop protocols for recording traits in seedstock herds and the automation of transfer of BBSE data to ABRI/breed societies, and
(iii) Uploading the data into BREEDPLAN for validation of genetic parameters and subsequent generation of Group EBVs for use by the industry to select high performing reproductive trait sires for use in improving the productivity and profitability of their herds. Some uploading of data (Scrotal circumference, Days to Calving, growth data etc) generated on Brahmans in this project has already occurred (Hans Graser - Final Beef CRC Forum, 12-14 June 2012).

6.2 Recommendations for on-going data collection and for new R&D

(i) As reported earlier, there is a need for the ongoing collection and recording of male and female traits within these Beef CRC research breeds and other major tropical breeds to determine the usefulness of these male and female reproductive traits evaluated in this project and to increase the accuracy of genetic correlation estimates between these male and female reproductive traits. In addition, there is also a need to establish the utility of measuring these bull traits in seedstock herds for selection programs by (a) Assessing the difficulty of measuring these traits at the industry level; and (b) Assessing the costs of data collection *versus* financial returns of using these traits.

(ii) With respect to semen traits in young bulls, there is a need for the routine collection of these traits in all young bulls in birth-year groups for both crush side and laboratory assessment and records to be uploaded onto the BREEDPLAN database – not just the records of sale bulls that pass the BBSE. Further, in these industry seedstock herds, about 5,000 phenotypes per breed for parameter estimation are required for the subsequent development of an EBV for a trait.

(iii) There is also a need for the development and implementation of a long term project 'to evaluate and demonstrate the economic benefits of selection for Lifetime Weaning Rate over a 10 year period in the major tropical breeds in

[71]Corbet and Burns (2012) Final Beef CRC Forum, 12-14 June 2012, Auditorium, Queensland Biosciences Precinct, University of Queensland, Carmody Road, St Lucia.

northern Australia, that is Brahman, Droughtmaster and Santa Gertrudis breeds.

(iv) Finally, there is an urgent need for a clearly developed genetic improvement and adoption strategy that focuses on a plan that encourages adoption and builds demand while always ensuring there is an adequate supply of a suitably labelled quality product to meet that demand.

7 Conclusions and recommendations

7.1 Conclusions

This project has identified -

(i) New male traits (PNS 18 and motility) that are heritable and genetically associated with scrotal circumference or female reproductive traits;
(ii) No antagonisms with other production traits;
(iii) Potential to use male traits for indirect selection to improve both male and female reproductive performance in northern Australian herds;
(iv) Genetic parameter estimates can change with age and can impact on development of recording protocols for genetic evaluation; and
(v) Potential value in seedstock producers recording BBSE for current herd and now a role for future herd (daughters).

7.2 Recommendations

(i) Distil all of the information from the Beef CRC projects NBP.361, 363 and 364 and focus on the key messages and outputs that will deliver improved reproductive performance by genetic selection.
(ii) Establish the relevant markers, prediction equations and EBVs and other key messages that have originated from the projects (and as identified by forum participants) and explore how they can be practically incorporated into implementation and adoption.
(iii) Identify the barriers to success of adoption and estimate the time required to achieve the objectives.
(iv) While motility has been identified as a useful trait in this project it was assessed by a limited number of highly skilled technical people (3 people) and the precision was good. If this trait is used widely throughout the industry as a predictor of male reproductive performance additional training and accreditation will be required by the veterinary profession.
(v) A review needs to be conducted on the poor adoption and uptake of quantitative genetics in the north compared to southern Australia as evidenced by the number of BREEDPLAN recorded calves in northern Australia and the number of sires for sale that lack BREEDPLAN data. The review should form the basis of proposed genetic extension and adoption strategies for northern Australia.
(vi) Evaluate and demonstrate the major improvements in profitability that can be achieved by selecting for improved reproductive performance. Such an outcome will instil confidence in the commercial sector that these genetic technologies will work, that they are cost effective and that they are achievable within the commercial sector.
(vii) Develop and implement a co-ordinated RD&E program to increase adoption of objective selection and genetic technologies in northern Australia to capitalise on Beef CRC findings.

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10 Appendices

10.1 Appendix I - Progress with Publication Plan – 30 June 2012

Submission date	Senior author	Working title or content	Target	Completed 30 June 2012
March 2011	Marina Fortes	Finding genes for economically important traits: Brahman cattle puberty	Conference – Applied Genomics for Sustainable Livestock Breeding, May 2011	Completed
March 2011	Marina Fortes	Sperm chromatin integrity was not influenced by pubertal development in cattle	Theriogenology, June 2011	Completed
March 2011	Marina Fortes	Principal component analysis in a population of Brahman bulls genotyped with a 50K SNP chip	AAABG Conference Proceedings July 2011	Completed
April 2011	Nick Corbet	Early-in-life traits of bulls as predictors of important male reproductive traits.	AAABG Conference Proceedings July 2011	Completed
April 2011	Jessica Crisp	Development of field protocols to measure GnRH induced LH response in tropical beef bull calves	Theriogenology	In preparation
April 2011	Michael McGowan	Recent advances in beef cattle reproduction – how science will improve herd performance	NABRUC August 2011	Completed
May 2011	Jessica Crisp	GnRH induced LH response in tropical beef bull calves	Theriogenology	In preparation
June 2011	Jessica Crisp	Relationship of GnRH induced LH response and male reproductive traits	Theriogenology	In preparation
July 2011	Jessica Crisp	Identification of seminal plasma proteins in Brahman bulls and their relationship with bull reproductive traits	Animal Reproduction Science	In preparation
August 2011	Jessica Crisp	Relationship between seminal and plasma 11βHSD and bull reproductive traits	Animal Reproduction Science	In preparation
September 2011	Jessica Crisp	PhD Thesis – Novel early life predictors of fertility in tropically adapted bulls	UQ	To be completed by end of 2013
November 2011	Brian Burns	Importance of sperm morphology as a phenotypic selection criteria in bulls	CRC Program 4 Distillation Workshop	Completed
March 2012	Brian Burns	General materials and methods paper describing environments, management practices, design and procedures	Animal Production Science	Internal review completed and submitted to journal
March 2012	Brian Burns	Male phenotypic results	Animal Production Science	Draft completed
March 2012	Nick Corbet	Male genetic parameters and genetic correlations	Animal Production Science	Internal review completed and submitted to journal
June 2012	Brian Burns	Final Report	Beef CRC and MLA	Submitted

10.2 Appendix II – List of project publications and presentations

Journal papers

Crisp JM, Anderson S, Burns BM, Holroyd RG, Boe-Hansen GB., McGowan MR.. (2012e, in preparation). Cortisol and cortisone levels seminal plasma and blood plasma levels in two year old tropically adapted beef bulls and their relationship to reproductive traits. *Animal Reproduction Science.*

Crisp JM, Moura, AA, Nouwens AS, Li Y, Venus BK, Burns BM, Holroyd RG, Boe-Hansen GB, McGowan MR (2012d, in preparation). Seminal Plasma Protein profiles of Brahman bulls and their relationship to reproductive traits. *Animal Reproduction Science*.

Crisp JM, Holroyd, R.G., McGowan, M., Playford, C., Boe-Hansen, G., Burns, B.M. (2012c, in preparation). The LH response to a GnRH challenge of Bos indicus bull calves and its relationship to reproductive traits measured until two years of age. *Theriogenology.*

Crisp JM, Holroyd RG, McGowan MR, Dawson K, Boe-Hansen GB, Burns BM (2012b, in preparation). LH response of pre-pubertal tropically adapted beef bull calves after a GnRH challenge. *Theriogenology.*

Crisp JM, Holroyd RG, McGowan MR, Doogan VJ, Playford C, Boe-Hansen GB, Burns BM (2012a, in preparation) Development of a field protocol for a two-sample test to measure GnRH induced LH response in beef bull calves in the tropics. *Theriogenology.*

Li Y, Burns BM, Corbet NJ, Corbet DH, Crisp LM, Venus BK, Johnston DJ, McGowan MR, Holroyd RG (2013, in review) Male traits and herd reproductive capability in tropical beef cattle. 4. Linear and non-linear phenotypic predictions for percent normal sperm at 24 months. *Animal Production Science*, [in preparation].

Burns BM, Corbet NJ, Corbet DH, Crisp JM, Venus BK, Johnston DJ, Li Y, McGowan MR, Holroyd RG (2013) Male traits and herd reproductive capability in tropical beef cattle. 3. Systematic evaluation of the effects of environmental and management on bull traits. *Animal Production Science*, [in preparation].

Fortes MRS, Sasazaki S, Kemper K, Reverter A, Pryce JE, Barendse W, Bunch R, McCulloch R, Harrison B, Bolormaa S, Zhang Y, Hawken RJ, Goddard ME, Lehnert SA (in preparation) Evidence for pleiotropism and recent selection in the *PLAG1* region in Australian Beef cattle.

Fortes MRS, Reverter A, Hawken RJ, Bolormaa S, Lehnert SA (in press) Candidate Genes Associated with Hormone Levels of Inhibin, Luteinising Hormone, and Insulin-like Growth Factor 1, Testicular Development and Sperm Quality in Brahman Bulls. *Biology of Reproduction*

Reverter A, Fortes MR (in press) Association Weight Matrix: A network-based approach towards functional genome-wide association studies. In: "Genome-Wide Association Studies, Humana Press, C Gondro, J van der Werf and B Hayes, eds.

Fortes MRS., Lehnert SA, Burns BM, Hawken R, Boe-Hansen GB, D'Atley K, Thomas M (2012) Genomic regions and quantitative trait loci associated with fertility traits in cattle: Advances from microsatellites to high-density chips. *Biology of Reproduction,* [In preparation].

Burns BM, Corbet NJ, Corbet, DH, Crisp JM, Venus BK, Johnston DJ, Li Y, McGowan MR, Holroyd RG (2012) Male traits and herd reproductive capability in tropical beef cattle. 1. Experimental design and animal measures. *Animal Production Science*, [Submitted].

Corbet NJ, Burns BM, Johnston DJ, Wolcott ML, Corbet DH, Venus BK, Li Y, McGowan MR, Holroyd RG (2012) Male traits and herd reproductive capability in tropical beef cattle. 2. Genetic parameters of bull traits. *Animal Production Science*, [Submitted].

Fortes MR, Li Y, Collis E, Zhang Y, Hawken RJ. (2012) The IGF1 pathway genes and their association with age of puberty in cattle. *Anim Genet.* 2012 May 4 . [Epub ahead of print]

Fortes MRS, Reverter A, Holroyd RG, Hawken RJ, Corbet NJ, Burns B, Fordyce G, Johnston D, Lehnert SA (2012) Candidate genes associated with inhibin, LH, IGF1, testicular development and sperm output in *Bos indicus* bulls. *Biology of Reproduction,* [Submitted].

Fortes MRS, Holroyd RG, Reverter A, Venus BK, Satake N, Boe-Hansen GB (2012) The integrity of sperm chromatin in young Tropical Composite bulls. *Theriogenology*, [In press].

Fortes MRS, Lehnert SA, Bolormaa S, Reich C, Fordyce G, Corbet NJ, Whan V, Hawken RJ, Reverter A (2012) Finding genes for economically important traits: Brahman cattle puberty. *Animal Production Science,* 52: 143-150.

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Johnston D J, Tier B., Graser H -U (2012) Beef cattle breeding in Australia with genomics: opportunities and needs. Anim. Prod. Sci. 52: 100-106

Snelling WM, Cushman RA, Fortes MR, Reverter A, Bennett GL, Keele JW, Kuehn LA, McDaneld TG, Thallman RM, Thomas MG. (2012) Physiology and Endocrinology Symposium: How single nucleotide polymorphism chips will advance our knowledge of factors controlling puberty and aid in selecting replacement beef females. *J Anim Sci.* **90**:1152-1165

Collis E, Fortes MR, Zhang Y, Tier B, Schutt K, Barendse W, Hawken R (2011) Genetic variants affecting meat and milk production traits appear to have effects on reproduction traits in cattle. *Anim Genet.* 2011 Oct 18. [Epub ahead of print]

Fortes MR, Reverter A, Nagaraj SH, Zhang Y, Jonsson NN, Barris W, Lehnert S, Boe-Hansen GB, Hawken RJ (2011) A single nucleotide polymorphism-derived regulatory gene network underlying puberty in 2 tropical breeds of beef cattle. *J Anim Sci.* **89**:1669-1683.

Meyer K, Tier, B (2011) "SNP Snappy": A Strategy for Fast Genome-Wide Association Studies Fitting a Full Mixed Model. *Genetics* 190: 275-277.

Burns BM, Gazzola C, Holroyd RG, Crisp J, McGowan MR (2011). Male reproductive traits and their relationship to reproductive traits in their female progeny: a systematic review. *Reproduction in Domestic Animals*, 46: 534-553.

Burns BM, Fordyce G, Holroyd RG (2010) Factors that impact on the capacity of beef cattle females to conceive, maintain a pregnancy and wean a calf – Implications for northern Australia: a review. *Animal Reproduction Science,* 122: 1-22.

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Burns BM, Holroyd RG (2006) Male indicator traits to improve female reproductive performance in tropical beef cattle genotypes. *The Australian Cattle Veterinarian,* 47: 14-15.

Beef CRC Requested Reviews

Burns BM, Gazzola C, McGowan MR (2005) Male indicator traits to improve female reproductive performance. Review for Beef CRC Scientific Review Committee, 7 November 2005.

Conference papers

Li Y, Corbet NJ, Burns BM, Corbet DH, Crisp JM, Venus BK, McGowan MR, Holroyd RG (2012) Potential bull traits for prediction of percent normal sperm at 24 months of age. Proceedings of the *International Society of Animal Genetics*, 33rd Conference, Cairns Convention Centre, North Queensland, 15-20 July, 2012. [Accepted].

Corbet NJ, Burns BM (2012) Genetics of Male Reproductive Traits in Tropical Beef Cattle. Final Beef CRC Forum, 12-14 June 2012, Auditorium, Queensland Biosciences Precinct, University of Queensland, Carmody Road, St Lucia.

Burns BM, Corbet NJ (2012) Beef CRC Male Fertility Research results in Northern Beef Herds. *The Bayer and Bioniche International Beef Cattle Genetics Conference*, Central Queensland University, Rockhampton, 6-7 May, 2012. pp. 10.

Fortes MR, Sazasaki S, Kemper K, Reverter A, Pryce J, Barendse W, Bunch R, Zhang Y, Hawken RJ, Goddard ME, Lehnert SA (2012) Mutations in the *PLAG1* region affect height, weight, puberty, IGF1 levels and fat deposition in beef cattle. Oral presentation at International Society for Animal Genetics Conference, Cairns, Australia July 2012.

Reverter A, Fortes MR, Bolormaa S, Zhang Y and Lehnert SA (2012) Accuracy of Genomic Selection for Fertility Traits in Australian Brahman Cattle. 4th ICQG conference Edinburgh, UK, June 2012.

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Burns BM (2009). Beef Cattle Genetic & Animal Breeding Activities in Northern Australia. BEEF AUSTRALIA 2009, '*FutureBeef: Smart Science, New Technologies, Profitable Beef Businesses'* Seminar, 5 May 2009, James Lawrence Pavillion, Rockhampton Showgrounds, Rockhampton Qld (Paper and Presentation).

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Holroyd RG (2008) Bull selection and management under tropical conditions. *3rd International Symposium on Applied Animal Reproduction*, (PS Baruselli and MM Seneda, editors,) Faculty of Veterinary Medicine and Zoology, University Sao Paulo, Londrina, Brazil, pp 68-77.

Mayes J.**,** Burns B, Holroyd R, Doogan V, McGowan M (2008) Timing of luteinising hormone (LH) response to various dose rates of gonadotrophin-releasing hormone (GnRH) stimulation in pre-pubertal Tropical Composite (TC) and Brahman (B) bull calves. *CRC Postgraduate Student Conference, Gold Coast Sea World Resort*, 4-7 November, 2008.

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Presentations

Corbet NJ (2012) Results of Beef CRC Male Fertility Project. Invited presentation to AgForce Meeting 29 June 2012, Namgooyah, Dingo, Central Queensland.

Corbet NJ Burns BM (2012) Genetics of Male Reproductive Traits in Tropical Beef Cattle. Final Beef CRC Forum, 12-14 June 2012, Auditorium, Queensland Biosciences Precinct, University of Queensland, Carmody Road, St Lucia.

Corbet NJ (2012) Bull Selection to Improve Fertility. Belmont Research Station Property Tour, BEEF AUSTRALIA 2012, 8 May 2012.

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Burns BM, Corbet NJ (2012) Beef CRC Male Fertility Research results in Northern Beef Herds. The Bayer and Bioniche International Beef Cattle Genetics Conference, Central Queensland University, Rockhampton, 6-7 May, 2012. pp. 10.

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Corbet NJ (2008) Beef CRC Bull Project Update. Presentation to DPI Extension Officers, Belmont Research Station Tour, 2 December, 2008.

Corbet NJ, Holroyd RG (2008) Beef CRC Male Indicator Traits Project – progress update. Presentation to producers at Mt. Eugene Field Day, Mt. Eugene, Jambin, Central Queensland, 21 August, 2008.

Industry communication

MLA Feedback magazine June 2012 issue: “Genomics boost Brahman fertility”.

Beef Bulletin Quarter 1 2012: “Tackling delayed puberty in Brahmans”.

PhD thesis

Marina Rufino Salinas Fortes: “Genes and Genetic Markers Associated with Puberty in Beef Cattle” The University of Queensland, School of Veterinary Science, May 2012.

10.3 Appendix III – List of Tabulated Results

Table I. Detailed description of traits measured on tropical breed bulls and their dams

Component Traits	Code	Description
Growth and carcass traits		
Live weight (kg)	LWT	Unfasted live weight using electronic weigh scales on the morning of the data collection date. Birth weight (LWT0) was recorded within 48hrs of parturition. Live weights were recorded at 6, 9, 12, 15, 18, 21 and 24 months of age.
Body condition score (1-5)	CS	Five-point scale with 1/3rd score increments adapted from the scale below reported by Upton *et al.* (2001) who cited the CS system of Lowman *et al*. (1976). to describe body reserves of fat and muscling 1 (poor) = The individual short ribs are sharp to touch and no tail head tissue can be felt. 2 (backward) = The individual short ribs can still be felt, but feel rounded rather than sharp. There is some tissue cover around the tail head. 3 (moderate) = The short ribs can only be felt with very firm thumb pressure. Areas either side of the tail head have some tissue cover that can be easily felt. 4 (prime) = The short ribs cannot be felt and tissue cover around the tail head is easily seen as slight mounds; folds of tissue are beginning to develop over the ribs and thighs of the animal 5 (fat) = The bone structure of the animal is no longer noticeable and the tail head is almost completely buried in body tissue. Folds of tissue are apparent over the ribs and thigh.
Hip Height (cm)	HH	Vertical distance from a fixed point to the top of the highest sacral vertebrae subtracted from the vertical distance from the fixed point to the ground at 15 months of age.
Rump fat (mm)	SP8	Real-time ultrasound-scanned subcutaneous fat depth at the P8 site (after “position 8” from the original research to define the optimum site for carcass fat measurement) on the rump (at the intersection of a line parallel to the spine from the *tuber ischium* and a line perpendicular to it from the spinous process of the third sacral vertebra); adapted from Upton *et al.* (1999; 2001).
Rib fat (mm)	SRIB	Real-time ultrasound-scanned subcutaneous fat depth between the 12th and 13th ribs; adapted from Upton *et al.* (1999; 2001).

Component Traits	Code	Description
Eye muscle area (cm^2)	SEMA	Real-time ultrasound-scanned cross-sectional area of the eye muscle (*M. longissimus thoracis et lumborum*) between the 12th and 13th ribs; adapted from Upton *et al.* (1999; 2001).
Adaptation traits		
Flight time (sec)	FT	Flight time was an electronically recorded time taken for an animal to cover a distance of approximately 2 m after exiting a weigh crush (Burrow *et al*. 1988). Flight times were recorded twice at weaning (FT6a and FT6b) at ~ 7 days apart (Burrow and Corbet 2000) and at 12, 18 and 24 months of age. Recorded by an experienced operator.
Rectal temperature (^oC)	RT	Rectal temperature measured with an Anritherm integrated thermometer (Anritherm HL600, Anritsu Meter Co. Ltd., Tokyo, Japan) and a rectal probe. Recorded by an experienced operator.
Time of rectal temperature (based on 24 hrs)	TRT	Time of the day when rectal temperature and BBSE were recorded.
Hormonal traits		
Inhibin (ng/mL)	IN4	At GnRH-stimulated LH Time 0, a basal whole blood sample (minimum 5mL) was collected by venipuncture from the jugular vein of restrained calves (3-4months of age - coincided with branding) into 10mL Serum BD Vacutainer® tubes (Becton, Dickinson and Company) using a 20G x 1" (0.9 x 25 mm) BD Vacutainer® Precision Glide™ needle (Becton, Dickinson and Company). Tubes were centrifuged crush side at 2500g for 20 min and the sera frozen at -20 °C until assayed for concentrations of inhibin. Sera were assayed by Monash University using established protocols (Phillips 2005).

Component Traits	Code	Description
GnRH-stimulated LH (ng/mL)	LH4	At Time 0, a basal whole blood sample (minimum 5mL) was collected by venipuncture from the jugular vein of restrained calves (3-4months of age - coincided with branding – basal blood LH4) into a 10mL Lithium Heparin BD Vacutainer® tubes (Becton, Dickinson and Company) using a 20G x 1" (0.9 x 25 mm) BD Vacutainer® Precision Glide™ needle (Becton, Dickinson and Company). Calves were treated immediately post-sampling with 0.5 µg/kg (intramuscular) injection of gonadotrophin releasing hormone (GnRH) (Fertagyl™, Intervet Australia Pty Limited). At 20min post-GnRH injection, the calves were restrained for a second time and a second whole blood sample (minimum 5mL) was collected by venipuncture from the jugular vein to establish the GnRH-stimulated LH blood level (stimulated blood LH4 level). This dose rate of 0.5 µg/kg of GnRH was considered sufficient to elicit a significant LH response when captured 20 min post-GnRH treatment. Calf crush order was identified/recorded by paint markings at the first sampling and the sampling order was maintained at the second blood sample. Tubes were centrifuged crush side at 2500g for 20 min and the plasma frozen at -20 °C until assayed for concentrations of LH. Plasma LH concentrations in all samples were measured by a double-antibody radioimmunoassay (RIA) procedure (Martin *et al*. 1980) that was modified by Hotzel *et al.* (1998) and Hawken *et al.* (2009) and by Ms. M. Blackberry, University of Western Australia. LH levels were recorded by the laboratory and returned to the Project Team for checking and entering onto the CRC database.
Insulin-like Growth Factor -I (ng/mL)	IGF6	At weaning (~6 months of age), whole blood was collected by venipuncture from the coccygeal (tail) vein of restrained calves, using a 20G x 1" (0.9 x 25 mm) BD Vacutainer® Precision Glide™ needle (Becton, Dickinson and Company), onto bloodspot collection cards supplied by PrimeGRO™ to determine blood IGF-I levels. IGF-I was assayed using a commercially available (Rivalea (Australia) Pty. Ltd.) enzyme-linked immunosorbent assay (Moore *et al.* 2005).
Conformation traits[A, B]		
Leg structure (1-9)	LStruct	A score of leg angularity on a scale of 1-9, with 9 being good and 1 for an animal that can hardly walk because of incorrect angularity, either too straight or too bent (Anon 1994).

Component Traits	Code	Description
	LCode	The letter code that goes with the number explains any problem - P (pastern) = Excessive angle at the pastern. T (straight hocks) = Insufficient angle at the hock when viewed from the side. S (sickle hocks) = Excessive angle at the hock when viewed from the side. B (bowed legs) = Bowed out at the hocks when viewed from behind. H (cow hocks) = Cow hocked or too close at the hocks when viewed from behind. C (stringhalt) = Suffering from sub-luxation of the patella (degree of severity should be recorded as mild, moderate or severe).
Foot structure (1-9)	FStruct	A score of feet structure on a scale of 1-9 with 9 being a perfect foot while 1 represents a foot that almost renders the animal a cripple (Anon 1994).
	FCode	The letter code that goes with the number explains any problem. L (length) = Undesirably long feet when viewed from the side (toes are too long). C (curve) = Excessive curvature of the claws when viewed from the front, ie, scissor claws (toes are too curved or clawed). H (heel) = feet that are very low in the heel.
Sheath score (1-9)	SH	A score of tightness and pendulousness of the sheath ranging from 1 being extremely large to 9 being very small (Anon 1994).
	SHCode	9 (tight) = Moderately tight sheath, fairly close to abdominal wall, depth up to about 10cm with obvious retractor prepuce muscle, moderate sized preputial opening. 7-8 (small) = Sheath hangs at 45^{o} angle, depth up to about 15cm, moderate umbilicus 5-6 (moderate) = Sheath hangs at 45^{o} angle, slightly more pendulous than 2, with depth less than 20cm, and larger umbilicus 3-4 (large) = Sheath hangs at up to 90^{o} angle, excessive looseness of umbilical area, with depth just above hock-knee horizontal line 1-2 (very large) = Sheath hangs at up to 90^{o} angle, excessive looseness and length of umbilicus, sheath depth at or below hock-knee horizontal line, often with eversion of the preputial mucosa.
Prepuce eversion (mm)	EV	An estimate (mm) of the length of preputial mucosa exposed when relaxed (Holroyd *et al.* 2002b).

Component Traits	Code	Description
Penis erection (yes/no) and structure	PE and PS	An erection of the penis was recorded if it occurred or did not occur. That is, whether the bull had an erection (y), just a penile protrusion (p) or no appearance (n) of the penis (Holroyd *et al.* (2002b). It is noted that an erection is an individual bull response to the electroejaculation procedure. The penis structure/anatomy was reported as visually normal or abnormal.
Horn status	HSt	Scored at branding time (~3-4 months of age). Each animal, where possible, was scored for the presence or absence of horns and also if the horn material was a scur (horn bud not attached), with a reassessment at 12-18 months of age P=Polled, S=Scurred, H=Horned.
Scrotal traits [A, B]		Recorded by experienced scorers – trained and supervised by an Australian Cattle Veterinarian (ACV) Accredited Examiner for Bull Breeding Soundness Evaluation (BBSE).
Scrotal circumference (cm)	SC	Scrotal circumference (SC) of the testicles at the widest diameter is still the best method of assessing testicular development (Barth 2000). Recommended SC measurement procedure with a standard metal tape (see Holroyd *et al.* 2002b; Entwistle and Fordyce 2003).
Testicular tone (1-5)	TT	Testicular tone is a score of the firmness and resilience of the testicles. On a scale of 1-5 with 1 = very soft, 3-4 ideal, 5 very hard – described by Holroyd *et al.* (2002b) and based on an ACV classification described by Entwistle and Fordyce (2003).
Semen collection traits[A, B]		
Volume (mL)	VOL	Volume recorded of a fresh ejaculate at bull body temperature used for sampling; the objective was to collect a minimum of 3mL of representative ejaculate. Volume recorded crush side immediately after semen collection.
Colour (1-5)	COL	Colour of a fresh ejaculate at bull body temperature scored on a scale of 1-5 with 1=pale, 2=cloudy to milky, 3=milky, 4=creamy, 5=yellow. Colour recorded crush side immediately after semen collection.
Density (1-5)	DENS	Density recorded on the ejaculate on a scale of 1-5 with 1=dilute or clear to cloudy, 2=cloudy to milky, 3=milky, 4=creamy, 5=thick creamy or dense. Density recorded crush side immediately after semen collection.

Component Traits	Code	Description
Mass activity (1-5)	MASS	Mass activity (or wave motion) scored within a fresh ejaculate at body temperature viewed at X10 on a scale of 1-5 with 1 being no swirl, 2-3 slow distinct swirl, 4 moderate swirl and 5 swirl is in continuous dark waves. Mass activity recorded crush side immediately after semen collection.
Motility (%)	MOT	Percentage of sperm viewed at X400 that are progressively motile by their own propulsion. Motility recorded crush side immediately after semen collection.
Sperm morphology traits[A]		For semen and sperm morphology traits, 0 denotes no ejaculate or a sperm-free ejaculate was collected and blank indicates no attempt was made to obtain an ejaculate. Immediately after each crush side evaluation of an ejaculate, a number of 50ul aliquots of ejaculate, dependent on the quality of/concentration of sperm/cells in the ejaculate, were taken with a micropipette and placed into 2.95ml of Phosphate Buffered Saline (PBS) for sperm morphology assessment (maximum of 5 drops). The sperm morphology assessment of every semen sample collected on every bull at 12, 18 and 24 month during this study was conducted by one of the authors of this paper. This experienced technician is an ACV Accredited sperm morphologist (research) and was responsible for scoring all normal and the range of abnormal sperm on each sample. Morphological assessment involves counting 100 cells systematically across a slide and noting the number of various abnormalities. Abnormalities are grouped into categories including head, midpiece, tail and droplets. A count of the normal cells allows per cent morphologically normal sperm to be calculated.
Knobbed acrosomes (%)	KA	The KA defect can be heritable due to a disturbance in testes thermoregulation (Entwistle and Fordyce 2003). If knobbed acrosomes are the only abnormality observed in an ejaculate where motility, volume and density are normal, the condition is probably genetic and will not improve. However, if motility, volume and density are poor and many other abnormalities are present, the condition is probably a sign of disturbed spermatogenesis caused by some stressor and the bull may recover.

Component Traits	Code	Description
Pyriform heads (%)	PH	The presence of a moderate number of PH in the absence of other signs of disturbed spermatogenesisis, is considered normal for some bulls (Entwistle and Fordyce 2003). However, when pronounced forms of pyriformity are observed, they usually are responsible for a decrease in fertility and are believed to result from a disturbance in spermatogenesis. Young bulls ≤2 years of age are more likely to recover from this condition than older bulls.
Abnormal mid-pieces (%)	MP	The abnormal sperm MP defect is the most common condition observed in bull ejaculates (Entwistle and Fordyce 2003). This defect may occur as an artefact due to prolonged contact with a hypotonic solution (Negrosin-Eosin stain), cold-shock or other environmental stressors. This type of abnormality can be common in some bulls and fluctuations in the percentage of affected spermatozoa can occur throughout the year. The prognosis of this condition varies with the circumstances and the presence of other types of abnormalities. If this defect is present in the absence of other abnormalities, this condition is usually transient in nature and recovery can occur within 16 days.
Proximal droplets (%)	PD	Entwistle and Fordyce (2003) reported that PD are normal in the pubertal bull and their incidence decreases with age. However, in the mature bull, these droplets can indicate abnormal spermiogenesis and/or epididymal function. These droplets can often be observed in conjunction with other abnormalities of the head and mitochondrial sheath.
Swollen acrosomes (%)	SA	The SA defect can be associated with a 'rusty load'/accumulated sperm condition (Entwistle and Fordyce 2003) (Table 3). The ageing of sperm causes the acrosome to undergo a similar reaction to capacitation which results in the lifting of the acrosome and the failure of the sperm to attach to the oocyte. This condition is often observed in conjunction with other head abnormalities such as knobbed acrosomes.
Abnormal tails and loose heads (%)	TH	The TH defect may occur as a result of temperature shock to the epididymis (Entwistle and Fordyce 2003). This condition is usually transient and the level of defects may decrease after 8-11 days.
Vacuoles and teratoids (%)	VT	The AT defect can occur during spermiogenesis and may be a result of extreme temperatures or stress (Entwistle and Fordyce 2003). Bulls can recover from this condition within 6 weeks of exposure to the insult, however, some bulls can be more susceptible to this condition and may not recover.

Component Traits	Code	Description
Morphologically normal sperm (%)	PNS	Percentage of sperm that have no morphological attributes known to be indicative of sub-fertility. See description of sperm morphology traits above. In a study conducted in tropical genotype bulls managed under extensive grazing conditions and in multiple-sire mated herds in northern Australia, PNS was shown to be the best practical measure and consistent predictor of bull fertility (Fitzpatrick *et al.* 2002; Holroyd *et al.* 2002*a*). Holroyd *et al*. (2002a) reported that sperm morphology accounted for 35-57% of the variation in calf output between bulls. Therefore, per cent morphologically normal sperm at 24 months (PNS24) of age was identified as the benchmark trait for male reproductive performance in this study as these bulls could not logistically be naturally mated to determine genetic parameters for individual calf-output.

[A] Each trait was measured according to the standards prescribed by the Australian Cattle Veterinarians (Entwistle and Fordyce 2003).

[B] Experienced scorers trained and supervised by an Australian Cattle Veterinarian (ACV) Accredited Examiner for Bull Breeding Soundness Evaluation (BBSE).

Table II. Summary of attrition due to culling and death of young bulls from weaning to 2 years of age

		Culls			Deaths			Total	Percent
Genotype	**Exit Age**	**Cryptorchid**	**Hypoplasia**	**Other**	**Injury**	**Sickness**	**Unknown**		**of genotype**
Brahman	6 to 12mths	11	4	3	0	6	13	37	
	13 to 18mths	4	5	0	0	3	5	17	
	19 to 24 mths	5	20	3	0	1	3	32	
	Total	20	29	6	0	10	21	86	5.7
Tropical Composite	6 to 12mths	11	5	4	3	5	7	35	
	13 to 18mths	1	3	0	0	3	6	13	
	19 to 24mths	9	11	4	2	2	8	36	
	Total	21	19	8	5	10	21	84	3.7
Crossbred	6 to 12mths	0	0	0	0	0	1	1	
	13 to 18mths	0	2	0	0	3	1	6	
	19 to 24mths	0	0	0	0	0	2	2	
	Total	0	2	0	0	3	4	9	3.6
Grand total		41	50	14	5	23	46	179	
Percent overall		1.0	1.2	0.3	0.1	0.6	1.1	4.4	

Cryptorchid, absence of one or both testes; hypoplasia, gross underdevelopment of one testicle; Other, culled due to injury, ill thrift or poor temperament; Unknown, cause of death not obvious

Table III. Summary statistics for growth, carcass and testicular measures within genotype

		Brahman						Tropical Composite					
Trait	**Unit**	**N**	**Min**	**Max**	**Mean**	**Std**	**CV**	**N**	**Min**	**Max**	**Mean**	**Std**	**CV**
Live weight													
Birth	kg	1473	20	59	35.3	5.77	16	2418	18	62	36.2	5.93	16
6 mth (kg)	kg	1639	104	323	203.7	33.51	16	2424	96	344	220.1	39.61	18
9 mth (kg)	kg	1490	110	323	217.2	34.95	16	2133	116	347	237.4	38.96	16
12 mth (kg)	kg	1469	125	360	246.9	35.27	14	2106	133	420	275.2	40.80	15
15 mth (kg)	kg	1462	144	430	297.4	38.43	13	2099	186	456	319.3	44.06	14
18 mth (kg)	kg	1436	214	488	353.2	38.36	11	2097	228	510	368.8	45.12	12
21 mth (kg)	kg	1432	225	540	365.1	42.70	12	2095	228	519	371.9	47.44	13
24 mth (kg)	kg	1430	222	570	383.9	44.35	12	2087	236	580	392.1	50.70	13
Body condition score													
9 mth	1-5	1421	1.3	3.3	2.4	0.33	14	1962	1.3	3.3	2.4	0.33	14
12 mth	1-5	1463	1.0	3.3	2.4	0.33	14	2102	1.3	3.3	2.4	0.33	14
15 mth	1-5	1415	1.0	3.3	2.5	0.28	11	2099	1.7	3.3	2.4	0.28	12
18 mth	1-5	1424	1.7	3.3	2.8	0.20	7	2095	1.7	3.3	2.7	0.28	10
21 mth	1-5	1424	1.0	3.3	2.7	0.27	10	2088	1.0	3.3	2.5	0.33	13
24 mth	1-5	1410	1.7	3.3	2.7	0.21	8	2078	1.0	3.3	2.5	0.31	12
Carcass													
Rib fat 15 mth (mm)	mm	1458	0.5	3.0	1.1	0.24	22	2099	0.5	3.0	1.0	0.14	14
Rump fat 15mth (mm)	mm	1458	0.5	5.0	1.4	0.56	40	2099	0.5	4.0	1.1	0.30	27
EMA 15mth	cm^2	1458	21	71	46.8	7.85	17	2097	21	77	50.7	8.11	16
Height of animal													
Hip height 15mth	cm	1457	110	144	128.0	4.89	4	2099	105	139	124.9	4.87	4
Scrotal circumference													
6 mth	cm	1609	12	25	17.2	1.71	10	2399	11	31	19.3	2.56	13
9 mth	cm	1361	13	33	19.1	2.67	14	1937	15	34	23.8	3.87	16
12 mth	cm	1448	13	35	21.2	3.13	15	2093	15	37	26.5	3.37	13
15 mth	cm	1108	16	40	24.7	3.73	15	1570	18	39	29.3	3.10	11
18 mth	cm	1409	16	42	26.4	3.49	13	2081	19	40	29.9	3.00	10
21 mth	cm	1411	19	41	28.5	3.26	11	2077	18	41	30.8	2.98	10
24 mth	cm	1403	19	42	30.2	3.21	11	2069	17	42	31.6	2.87	9
Testes tone (1-5)													
12 mth	1-5	1340	2	4	3.7	0.46	12	1924	2	5	3.86	0.37	10
18 mth	1-5	1410	2	4	3.9	0.35	9	2083	2	4	3.83	0.39	10
24 mth	1-5	1402	3	5	3.9	0.31	8	2069	2	5	3.85	0.37	10

N, number of animals recorded for each trait. Min and Max, minimum and maximum of the trait range. Std, standard deviation. CV, coefficient of variation is the Std expressed as a percentage of the mean. See Table 4 for trait description.

Table IV. Summary statistics for adaptation, hormonal and conformation traits within genotype

			Brahman						Tropical Composite					
Trait	**Age**	**Unit**	**N**	**Min**	**Max**	**Mean**	**Std**	**CV**	**N**	**Min**	**Max**	**Mean**	**Std**	**CV**
Adaptation traits														
Flight time	6 mth a[A]	sec	1619	0.24	5.40	1.20	0.63	53	2384	0.19	5.40	1.23	0.50	41
Flight time	6 mth b[A]	sec	1607	0.27	5.40	1.20	0.63	53	2274	0.39	5.40	1.23	0.55	45
Flight time	12 mth	sec	1465	0.45	6.67	1.80	0.85	47	2101	0.44	7.66	1.70	0.68	40
Flight time	18 mth	sec	1326	0.50	9.90	2.10	1.01	48	1924	0.57	9.90	2.10	0.84	40
Flight time	24 mth	sec	1429	0.51	7.02	2.10	0.83	40	2082	0.63	7.02	1.90	0.61	32
Rectal temperature	12 mth	°C	540	37.0	40.7	39.2	0.49	1	792	37.3	41.0	39.2	0.50	1
Rectal temperature	24 mth	°C	509	37.2	41.5	39.3	0.66	2	785	37.1	40.8	39.3	0.55	1
Hormonal traits														
GnRH-stimulated LH	4 mth	ng/mL	1025	0.19	29.34	5.21	4.46	86	1520	0.17	31.76	7.06	5.16	73
Inhibin	4 mth	ng/mL	1288	3.21	16.22	7.36	1.82	25	1895	2.66	15.05	7.82	1.92	25
IGF-I	6 mth	ng/mL	1626	56	1765	517	302	58	2415	47	1838	532	299	56
Conformation traits														
Sheath score	12 mth	1-9	1424	2	9	4.4	1.10	25	2071	1	9	6.9	1.77	26
Sheath score	18 mth	1-9	1437	1	8	4.3	1.19	28	2104	1	9	7.0	1.73	25
Sheath score	24 mth	1-9	1430	1	8	4.0	1.04	26	2091	1	9	6.8	1.74	26
Prepuce eversion	12 mth	mm	1362	0	100	11	16.6	151	1943	0	150	11	22.1	201
Prepuce eversion	18 mth	mm	1438	0	100	18	21.0	117	2104	0	120	10	20.9	209
Prepuce eversion	24 mth	mm	1430	0	150	26	25.6	98	2091	0	180	12	25.1	209
Leg structure	12 mth	1-9	1362	7	9	8.9	0.33	4	1946	7	9	8.9	0.30	3
Leg structure	18 mth	1-9	1329	6	9	8.9	0.34	4	1932	7	9	8.9	0.33	4
Leg structure	24 mth	1-9	1431	6	9	8.9	0.31	3	2091	6	9	8.9	0.30	3
Feet structure	12 mth	1-9	1350	5	9	8.5	0.63	7	1927	4	9	7.8	0.87	11
Feet structure	18 mth	1-9	1315	5	9	8.4	0.69	8	1921	4	9	8.0	0.80	10
Feet structure	24 mth	1-9	1401	4	9	8.4	0.66	8	2068	4	9	7.8	0.86	11

[A] Flight time was recorded twice at weaning (see Table 4) to derive a more reliable measure of genetic merit for flight time.

N, number of animals recorded for each trait. Min and Max, minimum and maximum of the trait range. Std, standard deviation. CV, coefficient of variation is the Std expressed as a percentage of the mean. See Table 4 for trait definition.

Table V. Summary statistics for semen and sperm morphology traits within genotype

			Brahman						Tropical Composite					
Trait	**Age**	**Unit**	**N**	**Min**	**Max**	**Mean**	**Std**	**CV**	**N**	**Min**	**Max**	**Mean**	**Std**	**CV**
Semen traits														
Ambient temperature[A]	12 mth	^{o}C	1361	17.0	41.0	30.5	4.49	15	1943	17.0	41.0	30.1	4.80	16
Ambient temperature	18 mth	^{o}C	1437	6.0	34.0	26.7	4.45	17	2103	4.0	34.0	26.0	4.96	19
Ambient temperature	24 mth	^{o}C	1429	16.0	40.0	29.1	4.23	15	2090	15.0	40.0	28.0	4.26	15
Volume	12 mth	mL	807	0.0	12.0	3.6	1.97	55	1843	0.0	14.0	5.1	2.36	46
Volume	18 mth	mL	1308	0.0	13.0	4.7	2.32	49	2058	0.5	14.0	5.7	2.35	41
Volume	24 mth	mL	1387	0.0	15.0	6.3	2.68	43	2058	0.0	18.0	6.1	2.74	45
Density	12 mth	1-5	753	0.5	4.0	1.7	0.75	44	1821	0.5	5.0	2.4	0.97	40
Density	18 mth	1-5	1264	0.5	5.0	2.2	0.95	43	2041	0.0	5.0	2.8	1.00	36
Density	24 mth	1-5	1389	0.0	5.0	3.1	0.87	28	2057	0.0	5.0	3.2	0.86	27
Mass activity	12 mth	1-5	754	0.0	4.0	0.4	0.75	188	1822	0.0	4.5	1.5	1.35	90
Mass activity	18 mth	1-5	1306	0.0	4.5	1.4	1.19	85	2062	0.0	5.0	2.2	1.24	56
Mass activity	24 mth	1-5	1390	0.0	5.0	2.5	1.14	46	2060	0.0	5.0	2.8	1.06	38
Motility	12 mth	%	754	0	90	16	26.1	163	1821	0	95	46	33.9	73
Motility	18 mth	%	1306	0	98	41	30.8	75	2064	0	100	57	28.1	49
Motility	24 mth	%	1390	0	98	67	25.4	38	2060	0	98	70	24.3	35
Sperm morphology														
Normal sperm	12 mth	%	103	2	87	23	20.1	87	968	1	96	55	27.9	51
Normal sperm	18 mth	%	826	0	98	49	29.1	59	1794	0	97	67	22.6	34
Normal sperm	24 mth	%	1235	1	98	72	23.1	32	1912	0	99	75	19.1	25
Knobbed acrosomes	12 mth	%	103	0	13	1	2.3	153	968	0	64	2	4.3	268
Knobbed acrosomes	18 mth	%	826	0	52	1	3.1	281	1794	0	70	1	4.3	358
Knobbed acrosomes	24 mth	%	1235	0	32	1	2.3	288	1912	0	82	1	4.1	410
Abnormal mid-pieces	12 mth	%	103	1	60	19	12.3	64	968	0	83	14	12.7	91
Abnormal mid-pieces	18 mth	%	826	0	74	15	12.3	82	1794	0	77	13	11.7	90
Abnormal mid-pieces	24 mth	%	1235	0	87	11	12.3	109	1912	0	89	10	10.3	103

Table V. Summary statistics for semen and sperm morphology traits within genotype (continued)

Trait	Age	Unit	N	Min	Max	Mean	Std	CV	N	Min	Max	Mean	Std	CV
Sperm morphology														
Proximal droplets	12 mth	%	103	1	88	44	23.2	53	968	0	96	19	22.6	118
Proximal droplets	18 mth	%	826	0	91	25	26.7	107	1794	0	82	7	11.5	169
Proximal droplets	24 mth	%	1235	0	90	8	15.6	195	1912	0	81	4	7.5	178
Pyriform heads	12 mth	%	103	0	10	1	1.9	173	968	0	44	1	2.0	286
Pyriform heads	18 mth	%	826	0	16	0	1.2	240	1794	0	19	0	1.2	240
Pyriform heads	24 mth	%	1235	0	16	0	0.8	400	1912	0	28	0	1.2	300
Swollen acrosomes	12 mth	%	103	0	18	1	2.4	218	968	0	21	1	2.0	222
Swollen acrosomes	18 mth	%	826	0	27	1	2.0	200	1794	0	25	1	2.4	218
Swollen acrosomes	24 mth	%	1235	0	24	1	1.8	225	1912	0	79	1	2.6	325
Abnormal tails, heads	12 mth	%	103	0	32	5	6.4	128	968	0	75	6	8.5	142
Abnormal tails, heads	18 mth	%	826	0	75	6	9.2	151	1794	0	98	8	11.4	143
Abnormal tails, heads	24 mth	%	1235	0	72	6	9.0	161	1912	0	92	6	10.2	165
Vacuoles and teratoids	12 mth	%	103	0	56	8	10.4	125	968	0	64	4	7.0	171
Vacuoles and teratoids	18 mth	%	826	0	84	5	9.6	178	1794	0	100	4	7.0	194
Vacuoles and teratoids	24 mth	%	1235	0	86	3	7.3	243	1912	0	100	3	6.1	226

[A] Ambient temperature is not a trait of the animal but was recorded at time of BBSE to investigate effects on semen traits and rectal temperature

N, number of animals recorded for each trait. Min and Max, minimum and maximum of the trait range. Std, standard deviation. CV, coefficient of variation is the Std expressed as a percentage of the mean. See Table 4 for trait definition.

Table VI. Additive variance (σ^2_a), phenotypic variance (σ^2_p) and heritability (h^2) of blood hormone levels and production traits of Brahman and Tropical Composite bulls

	Brahman			Tropical Composite		
Trait	σ^2_a	σ^2_p	h^2	σ^2_a	σ^2_p	h^2
LH4	4.15	13.29	0.31 (0.10)	7.50	15.50	0.48 (0.08)
IN4	2.09	2.84	0.74 (0.09)	2.15	2.97	0.72 (0.10)
IGF6	7237	16579	0.44 (0.08)	6266	17533	0.36 (0.07)
FT6	0.078	0.277	0.28 (0.07)	0.078	0.254	0.31 (0.07)
RT12	0.051	0.174	0.29 (0.13)	0.028	0.166	0.17 (0.09)
WT15	244.6	626.1	0.39 (0.10)	542.7	876.6	0.62 (0.10)
CS15	0.010	0.048	0.21 (0.07)	0.012	0.051	0.23 (0.06)
P815	0.114	0.289	0.39 (0.09)	0.008	0.083	0.10 (0.04)
EMA15	10.1	27.7	0.37 (0.08)	16.6	32.2	0.52 (0.07)
HH15	5.97	13.11	0.46 (0.09)	8.46	15.24	0.56 (0.07)
SH18	0.293	0.986	0.30 (0.08)	0.807	2.327	0.35 (0.08)
EV18	126.3	419.0	0.30 (0.08)	100.3	428.8	0.23 (0.06)

See Table I for trait description; approximate standard error shown in parentheses

Table VII. Additive variance (σ^2_a), phenotypic variance (σ^2_p) and heritability (h^2) of scrotal circumference of Brahman and Tropical Composite bulls

		Brahman			Tropical Composite		
Trait		σ^2_a	σ^2_p	h^2	σ^2_a	σ^2_p	h^2
SC6		0.81	1.75	0.46 (0.08)	1.44	3.50	0.41 (0.08)
SC6	*Wt. adj.*	0.51	1.45	0.35 (0.07)	1.16	2.78	0.42 (0.07)
SC12		3.07	4.72	0.65 (0.08)	3.42	7.47	0.46 (0.09)
SC12	*Wt. adj.*	2.52	3.86	0.65 (0.08)	2.77	6.24	0.44 (0.08)
SC18		5.06	6.76	0.75 (0.09)	3.10	7.25	0.43 (0.09)
SC18	*Wt. adj.*	4.40	5.89	0.75 (0.08)	2.63	6.25	0.42 (0.08)
SC24		4.71	6.31	0.75 (0.09)	2.98	6.73	0.44 (0.09)
SC24	*Wt. adj.*	3.81	5.18	0.74 (0.09)	2.74	5.86	0.47 (0.09)

See Table I for trait description. Measurements were made from weaning to 24 months of age; variance components are shown with (*Wt. adj.*) and without body weight as a covariate for each trait; approximate standard error shown in parentheses

Table VIII. Additive variance (σ^2_a), phenotypic variance (σ^2_p) and heritability (h^2) of semen quality traits of Brahman and Tropical Composite bulls

	Brahman			Tropical Composite		
Trait	σ^2_a	σ^2_p	h^2	σ^2_a	σ^2_p	h^2
12mths						
MASS12	0.147	0.217	0.68 (0.10)	0.511	1.528	0.33 (0.06)
MOT12	149.3	335.9	0.44 (0.09)	346.3	1073.0	0.32 (0.06)
PNS12	0.001	379.4	0.00 (0.00)	296.7	720.5	0.41 (0.10)
18mths						
MASS18	0.265	1.115	0.24 (0.07)	0.190	1.431	0.13 (0.05)
MOT18	123.9	804.9	0.15 (0.06)	116.4	768.3	0.15 (0.05)
PNS18	198.5	800.9	0.25 (0.09)	96.7	480.5	0.20 (0.06)
24mths						
MASS24	0.106	1.140	0.09 (0.05)	0.050	1.009	0.05 (0.03)
MOT24	30.3	608.4	0.05 (0.04)	53.4	558.6	0.10 (0.04)
PNS24	75.0	496.8	0.15 (0.06)	96.8	360.4	0.27 (0.06)

See Table I for trait description. Measurements were made at 12, 18 and 24 months of age; approximate standard error shown in parentheses

Table IX. Genetic and phenotypic correlations among hormone and production traits for Brahman bulls

Trait	LH4	IN4	IGF6	FT6	RT12	WT15	CS15	P815	EMA15	HH15	SH18	EV18
LH4	-	0.06 (0.17)	0.31 (0.18)	-0.24 (0.20)	-0.29 (0.26)	0.18 (0.19)	0.03 (0.24)	0.12 (0.19)	-0.15 (0.20)	-0.14 (0.18)	0.09 (0.22)	-0.02 (0.21)
IN4	0.00	-	0.36 (0.11)	-0.08 (0.15)	0.07 (0.22)	0.24 (0.13)	-0.12 (0.17)	0.13 (0.14)	0.19 (0.14)	0.13 (0.13)	-0.06 (0.15)	0.07 (0.14)
IGF6	0.11	0.15	-	-0.06 (0.16)	-0.33 (0.22)	0.53 (0.11)	0.24 (0.19)	-0.42 (0.14)	0.46 (0.13)	0.31 (0.13)	-0.30 (0.16)	0.23 (0.16)
FT6	0.00	0.01	0.02	-	-0.27 (0.25)	0.24 (0.17)	0.31 (0.19)	0.25 (0.17)	-0.04 (0.18)	-0.02 (0.17)	-0.05 (0.19)	0.10 (0.18)
RT12	-0.06	0.03	-0.09	-0.06	-	-0.47 (0.22)	0.19 (0.30)	0.10 (0.25)	-0.08 (0.25)	-0.12 (0.23)	0.05 (0.28)	0.24 (0.27)
WT15	0.05	0.06	0.16	0.06	-0.10	-	-0.10 (0.20)	-0.16 (0.16)	0.51 (0.12)	0.72 (0.08)	-0.20 (0.17)	0.08 (0.17)
CS15	0.00	-0.03	0.08	0.07	0.00	0.20	-	0.37 (0.17)	0.57 (0.16)	-0.48 (0.15)	-0.22 (0.20)	0.30 (0.21)
P815	-0.05	0.00	-0.08	0.04	-0.06	0.10	0.25	-	-0.11 (0.16)	-0.30 (0.14)	0.17 (0.17)	-0.08 (0.17)
EMA15	0.06	0.04	0.19	0.00	0.02	0.52	0.31	0.08	-	0.31 (0.14)	-0.42 (0.16)	0.20 (0.17)
HH15	-0.01	0.06	0.09	0.00	-0.04	0.64	-0.06	-0.03	0.27	-	-0.28 (0.16)	0.13 (0.16)
SH18	0.09	0.03	0.03	0.00	-0.01	-0.05	0.01	0.04	-0.02	-0.05	-	-0.67 (0.11)
EV18	-0.01	0.02	0.03	-0.05	0.02	0.05	-0.01	-0.03	0.03	0.07	-0.37	-

See Table I for trait description. Genetic correlations above the diagonal, phenotypic below; all estimates from bivariate analyses; approximate standard errors in parentheses; standard errors for phenotypic correlations ranged from 0.02 to 0.04; traits were measured between 4 and 18 months of age

Table X. Genetic and phenotypic correlations among hormone and production traits for Tropical Composite bulls

Trait	LH4	IN4	IGF6	FT6	RT12	WT15	CS15	P815	EMA15	HH15	SH18	EV18
LH4	-	0.14 (0.12)	0.23 (0.14)	0.14 (0.15)	-0.02 (0.29)	0.13 (0.13)	0.01 (0.17)	-0.29 (0.21)	0.17 (0.13)	-0.33 (0.20)	0.36 (0.14)	-0.34 (0.16)
IN4	0.01	-	0.12 (0.11)	0.09 (0.12)	-0.97 (0.34)	0.17 (0.10)	0.12 (0.14)	0.25 (0.18)	0.12 (0.11)	0.13 (0.13)	-0.28 (0.12)	0.28 (0.13)
IGF6	0.08	0.02	-	0.07 (0.14)	0.22 (0.22)	0.19 (0.09)	-0.04 (0.14)	-0.18 (0.18)	0.34 (0.10)	0.08 (0.09)	-0.04 (0.10)	0.00 (0.11)
FT6	-0.02	0.01	-0.04	-	0.06 (0.26)	0.15 (0.13)	0.19 (0.16)	-0.21 (0.21)	0.11 (0.13)	0.02 (0.17)	-0.08 (0.15)	0.25 (0.16)
RT12	-0.03	-0.08	0.01	-0.04	-	0.11 (0.26)	-0.34 (0.32)	-0.46 (0.34)	0.00 (0.26)	-0.08 (0.24)	0.08 (0.31)	0.03 (0.31)
WT15	0.00	0.08	0.11	0.00	0.05	-	0.20 (0.14)	-0.14 (0.18)	0.59 (0.07)	0.72 (0.08)	-0.27 (0.12)	0.34 (0.13)
CS15	-0.03	-0.02	0.04	-0.02	0.01	0.17	-	0.30 (0.20)	0.35 (0.13)	-0.46 (0.15)	0.17 (0.16)	0.00 (0.17)
P815	-0.06	0.00	-0.02	-0.01	-0.06	0.08	0.21	-	-0.24 (0.18)	-0.29 (0.15)	-0.07 (0.21)	0.32 (0.21)
EMA15	0.06	0.00	0.20	-0.04	0.03	0.48	0.26	0.02	-	0.34 (0.14)	0.21 (0.13)	-0.06 (0.14)
HH15	-0.02	0.06	-0.01	0.00	0.00	0.64	-0.07	-0.03	0.27	-	-0.29 (0.16)	0.11 (0.16)
SH18	0.13	-0.16	0.09	-0.02	-0.03	-0.11	0.06	-0.02	0.10	-0.05	-	-0.93 (0.03)
EV18	-0.10	0.12	-0.06	0.02	0.02	0.10	-0.04	0.03	-0.03	0.07	-0.55	-

See Table I for trait description. Genetic correlations above the diagonal, phenotypic below; all estimates from bivariate analyses; approximate standard errors in parentheses; standard errors for phenotypic correlations ranged from 0.02 to 0.04; traits were measured between 4 and 18 months of age

Table XI. Genetic and phenotypic correlations among scrotal circumference and semen quality traits for Brahman bulls

Trait	SC6	SC12	MASS12	MOT12	PNS12 [A]	SC18	MASS18	MOT18	PNS18	SC24	MASS24	MOT24	PNS24
SC6	-	0.69 (0.08)	0.16 (0.13)	0.13 (0.15)	-	0.57 (0.09)	-0.08 (0.18)	-0.09 (0.20)	-0.30 (0.19)	0.66 (0.08)	-0.24 (0.24)	0.00 (0.31)	-0.26 (0.22
SC12	0.42	-	0.66 (0.08)	0.70 (0.08)	-	0.92 (0.03)	0.69 (0.11)	0.65 (0.13)	0.31 (0.17)	0.83 (0.05)	0.51 (0.22)	0.71 (0.37)	0.23 (0.18
MASS12	0.06	0.43	-	0.99 (0.01)	-	0.52 (0.10)	0.79 (0.09)	0.73 (0.12)	0.43 (0.17)	0.30 (0.12)	0.66 (0.21)	0.58 (0.29)	0.45 (0.18
MOT12	0.06	0.47	0.76	-	-	0.55 (0.10)	0.74 (0.11)	0.73 (0.13)	0.48 (0.16)	0.36 (0.12)	0.69 (0.20)	0.62 (0.29)	0.44 (0.18
PNS12	-	-	-	-	-	-	-	-	-	-	-	-	-
SC18	0.37	0.77	0.31	0.35	-	-	0.82 (0.08)	0.79 (0.10)	0.50 (0.13)	0.97 (0.01)	0.75 (0.18)	0.88 (0.35)	0.32 (0.18
MASS18	0.02	0.36	0.28	0.31	-	0.48	-	0.97 (0.04)	0.91 (0.09)	0.70 (0.11)	1.00 (0.13)	1.00 (0.40)	0.60 (0.19
MOT18	0.01	0.27	0.20	0.22	-	0.38	0.78	-	0.86 (0.13)	0.66 (0.13)	0.99 (0.16)	1.00 (0.29)	0.76 (0.13
PNS18	0.05	0.23	0.19	0.25	-	0.31	0.40	0.28	-	0.31 (0.17)	0.73 (0.22)	0.84 (0.37)	0.93 (0.13
SC24	0.39	0.62	0.13	0.18	-	0.83	0.33	0.27	0.14	-	0.76 (0.17)	0.86 (0.31)	0.22 (0.19
MASS24	0.00	0.16	0.11	0.12	-	0.25	0.30	0.27	0.20	0.24	-	1.00 (0.13)	0.42 (0.30
MOT24	0.02	0.10	0.06	0.07	-	0.19	0.21	0.24	0.12	0.20	0.77	-	0.29 (0.40
PNS24	-0.01	0.06	0.07	0.08	-	0.15	0.27	0.33	0.32	0.12	0.31	0.32	-

[A] Correlations with percent normal sperm at 12 months in Brahmans were not estimable due to a small number of observations (n = 103) and no residual variance

See Table I for trait description. Genetic correlations above the diagonal, phenotypic below; all estimates from bivariate analyses; approximate standard errors in parentheses; standard errors for phenotypic correlations ranged from 0.02 to 0.04; semen quality was evaluated at 12, 18 and 24 months of age

Table XII. Genetic and phenotypic correlations among scrotal circumference and semen quality traits for Tropical Composite bulls

Trait	SC6	SC12	MASS12	MOT12	PNS12	SC18	MASS18	MOT18	PNS18	SC24	MASS24	MOT24	PNS24
SC6	-	0.87 (0.04)	0.18 (0.13)	0.16 (0.13)	0.30 (0.15)	0.87 (0.04)	0.43 (0.17)	0.30 (0.16)	0.32 (0.16)	0.86 (0.04)	0.33 (0.23)	0.29 (0.19)	0.38 (0.14)
SC12	0.54	-	0.60 (0.10)	0.56 (0.10)	0.55 (0.13)	0.97 (0.01)	0.85 (0.12)	0.55 (0.13)	0.35 (0.15)	0.92 (0.02)	0.62 (0.21)	0.42 (0.18)	0.35 (0.14)
MASS12	0.14	0.43	-	0.98 (0.01)	0.87 (0.08)	0.38 (0.12)	1.00 (0.08)	0.79 (0.10)	0.79 (0.11)	0.10 (0.14)	0.76 (0.20)	0.53 (0.17)	0.54 (0.13)
MOT12	0.14	0.42	0.84	-	0.77 (0.09)	0.35 (0.13)	1.00 (0.07)	0.85 (0.09)	0.79 (0.10)	0.10 (0.14)	0.78 (0.20)	0.55 (0.18)	0.47 (0.14)
PNS12	0.12	0.31	0.52	0.56	-	0.25 (0.15)	0.43 (0.20)	0.29 (0.19)	0.85 (0.10)	0.14 (0.16)	0.14 (0.29)	0.21 (0.23)	0.60 (0.14)
SC18	0.54	0.85	0.28	0.28	0.13	-	0.65 (0.14)	0.44 (0.15)	0.21 (0.16)	0.99 (0.01)	0.66 (0.18)	0.46 (0.17)	0.34 (0.14)
MASS18	0.10	0.29	0.28	0.30	0.17	0.29	-	0.89 (0.06)	0.74 (0.14)	0.31 (0.17)	0.98 (0.16)	0.84 (0.14)	0.54 (0.16)
MOT18	0.08	0.23	0.23	0.26	0.16	0.24	0.78	-	0.80 (0.12)	0.24 (0.16)	0.97 (0.17)	0.95 (0.12)	0.61 (0.14)
PNS18	0.08	0.23	0.28	0.31	0.44	0.22	0.32	0.40	-	0.04 (0.16)	0.80 (0.23)	0.83 (0.15)	0.98 (0.05)
SC24	0.53	0.76	0.17	0.17	0.04	0.90	0.19	0.17	0.10	-	0.55 (0.20)	0.33 (0.18)	0.20 (0.14)
MASS24	0.11	0.20	0.23	0.23	0.11	0.23	0.27	0.29	0.21	0.22	-	0.98 (0.08)	0.80 (0.18)
MOT24	0.08	0.15	0.19	0.20	0.14	0.17	0.25	0.29	0.28	0.17	0.80	-	0.76 (0.12)
PNS24	0.09	0.19	0.20	0.21	0.35	0.16	0.25	0.27	0.55	0.13	0.34	0.47	-

See Table 2 for trait description. Genetic correlations above the diagonal, phenotypic below; all estimates from bivariate analyses; approximate standard errors in parentheses; standard errors for phenotypic correlations ranged from 0.02 to 0.04; semen quality was evaluated at 12, 18 and 24 months of age

Table XIII. Genetic correlations of hormone and production traits with scrotal circumference and semen quality traits for Brahman bulls

Trait	SC6	SC12	MASS12	MOT12	PNS12 [A]	SC18	MASS18	MOT18	PNS18	SC24	MASS24	MOT24	PNS24
LH4	-0.11 (0.18)	0.02 (0.17)	0.25 (0.17)	0.26 (0.19)	-	0.03 (0.17)	0.23 (0.23)	0.08 (0.26)	-0.01 (0.26)	-0.11 (0.17)	0.39 (0.30)	0.12 (0.40)	0.27 (0.27
IN4	0.54 (0.10)	0.37 (0.11)	0.11 (0.12)	0.13 (0.14)	-	0.39 (0.10)	0.12 (0.17)	0.23 (0.18)	-0.09 (0.20)	0.39 (0.10)	0.16 (0.23)	0.00 (0.29)	-0.37 (0.18
IGF6	0.51 (0.11)	0.56 (0.09)	0.34 (0.13)	0.37 (0.13)	-	0.49 (0.10)	0.48 (0.15)	0.35 (0.19)	0.10 (0.21)	0.46 (0.11)	0.18 (0.24)	-0.01 (0.31)	0.44 (0.20
FT6	0.07 (0.16)	0.10 (0.15)	-0.30 (0.15)	-0.20 (0.17)	-	0.22 (0.14)	0.43 (0.18)	0.44 (0.21)	0.14 (0.23)	0.21 (0.14)	0.27 (0.28)	0.69 (0.36)	-0.26 (0.23
RT12	-0.10 (0.25)	-0.32 (0.20)	-0.24 (0.21)	-0.23 (0.23)	-	-0.40 (0.19)	-0.61 (0.24)	-0.31 (0.30)	-0.73 (0.23)	-0.30 (0.21)	-0.30 (0.38)	-0.32 (0.50)	0.04 (0.35
WT15	0.64 (0.09)	0.43 (0.11)	0.02 (0.15)	0.02 (0.16)	-	0.35 (0.11)	0.03 (0.19)	-0.06 (0.21)	-0.16 (0.22)	0.43 (0.11)	0.17 (0.25)	0.26 (0.32)	0.05 (0.23
CS15	-0.21 (0.18)	-0.03 (0.17)	-0.03 (0.18)	-0.16 (0.19)	-	-0.01 (0.16)	0.56 (0.18)	0.46 (0.23)	0.36 (0.23)	-0.02 (0.17)	0.04 (0.30)	0.21 (0.37)	0.69 (0.20
P815	-0.13 (0.15)	-0.21 (0.13)	-0.03 (0.15)	-0.18 (0.16)	-	-0.05 (0.13)	0.25 (0.18)	0.24 (0.20)	0.25 (0.20)	-0.07 (0.13)	0.32 (0.24)	0.48 (0.28)	0.27 (0.21
EMA15	0.20 (0.15)	0.16 (0.14)	-0.05 (0.15)	-0.08 (0.17)	-	0.14 (0.13)	0.13 (0.19)	-0.01 (0.22)	0.24 (0.21)	0.07 (0.14)	0.00 (0.26)	0.04 (0.32)	0.16 (0.23
HH15	0.51 (0.10)	0.18 (0.12)	-0.01 (0.13)	0.02 (0.14)	-	0.14 (0.11)	-0.30 (0.17)	-0.32 (0.19)	-0.04 (0.20)	0.25 (0.11)	-0.04 (0.24)	0.08 (0.30)	-0.14 (0.21
SH18	-0.12 (0.16)	0.23 (0.14)	0.33 (0.15)	0.37 (0.17)	-	0.14 (0.14)	0.29 (0.20)	0.56 (0.21)	0.12 (0.23)	-0.02 (0.15)	0.12 (0.27)	-0.05 (0.33)	0.18 (0.23
EV18	0.03 (0.15)	0.00 (0.14)	-0.15 (0.15)	-0.15 (0.16)	-	-0.03 (0.14)	-0.06 (0.19)	0.03 (0.21)	0.17 (0.23)	0.03 (0.14)	0.08 (0.26)	0.10 (0.32)	0.20 (0.22

[A] Correlations with percent normal sperm at 12 months in Brahmans were not estimable due to a small number of observations (*n* = 103) and no residual variance
See Table I for trait description. All estimates from bivariate analyses; approximate standard errors in parentheses; semen quality was evaluated at 12, 18 and 24 months of age

Table XIV. Genetic correlations of hormone and production traits with scrotal circumference and semen quality traits for Tropical Composite bulls

Trait	SC6	SC12	MASS12	MOT12	PNS12	SC18	MASS18	MOT18	PNS18	SC24	MASS24	MOT24	PNS24
LH4	0.20 (0.14)	0.28 (0.14)	0.04 (0.16)	0.20 (0.15)	-0.40 (0.16)	0.18 (0.15)	0.44 (0.19)	0.34 (0.18)	-0.20 (0.18)	0.17 (0.15)	0.52 (0.29)	0.37 (0.22)	-0.10 (0.17
IN4	0.18 (0.11)	0.28 (0.11)	-0.33 (0.12)	-0.31 (0.12)	-0.03 (0.15)	0.35 (0.10)	-0.18 (0.17)	-0.15 (0.16)	-0.20 (0.15)	0.40 (0.10)	-0.17 (0.25)	-0.17 (0.19)	-0.26 (0.13
IGF6	0.38 (0.11)	0.42 (0.11)	0.23 (0.14)	0.15 (0.14)	0.11 (0.16)	0.28 (0.12)	0.37 (0.18)	0.21 (0.17)	0.04 (0.17)	0.19 (0.13)	0.28 (0.24)	0.09 (0.20)	-0.01 (0.15
FT6	0.30 (0.13)	0.39 (0.13)	-0.03 (0.15)	-0.11 (0.15)	0.01 (0.17)	0.34 (0.14)	0.32 (0.19)	0.10 (0.18)	-0.12 (0.17)	0.38 (0.13)	-0.15 (0.27)	0.06 (0.22)	-0.02 (0.16
RT12	-0.25 (0.27)	-0.37 (0.26)	0.10 (0.29)	0.11 (0.30)	-0.16 (0.32)	-0.24 (0.27)	0.01 (0.38)	-0.14 (0.35)	-0.39 (0.30)	-0.17 (0.29)	-0.46 (0.53)	-0.35 (0.40)	-0.15 (0.31
WT15	0.68 (0.06)	0.57 (0.07)	-0.11 (0.13)	0.00 (0.13)	-0.06 (0.14)	0.66 (0.06)	0.01 (0.17)	0.04 (0.16)	-0.14 (0.15)	0.68 (0.06)	0.40 (0.22)	0.38 (0.17)	-0.05 (0.14
CS15	-0.03 (0.14)	0.07 (0.15)	-0.04 (0.16)	0.00 (0.16)	-0.07 (0.18)	0.09 (0.15)	-0.04 (0.21)	-0.13 (0.20)	0.12 (0.18)	0.08 (0.15)	-0.14 (0.28)	0.03 (0.22)	0.03 (0.17
P815	-0.26 (0.18)	-0.13 (0.19)	-0.19 (0.21)	-0.21 (0.21)	0.08 (0.22)	-0.01 (0.19)	-0.05 (0.26)	0.14 (0.24)	0.04 (0.23)	0.08 (0.19)	0.17 (0.35)	0.15 (0.27)	0.13 (0.21
EMA15	0.32 (0.11)	0.09 (0.12)	-0.03 (0.13)	-0.04 (0.13)	-0.07 (0.15)	0.25 (0.12)	-0.14 (0.17)	-0.17 (0.16)	-0.02 (0.15)	0.24 (0.12)	0.16 (0.23)	0.13 (0.19)	-0.04 (0.14
HH15	0.51 (0.11)	0.20 (0.12)	-0.05 (0.14)	-0.01 (0.15)	-0.12 (0.15)	0.14 (0.12)	-0.31 (0.17)	-0.33 (0.19)	-0.03 (0.21)	0.29 (0.11)	-0.03 (0.25)	0.08 (0.31)	-0.17 (0.21
SH18	-0.46 (0.11)	-0.29 (0.13)	0.39 (0.14)	0.37 (0.14)	0.11 (0.17)	-0.47 (0.10)	0.24 (0.20)	0.10 (0.19)	0.12 (0.18)	-0.56 (0.09)	0.01 (0.28)	-0.10 (0.22)	-0.16 (0.16
EV18	0.40 (0.12)	0.08 (0.15)	-0.42 (0.14)	-0.38 (0.15)	-0.17 (0.18)	0.25 (0.14)	-0.42 (0.19)	-0.34 (0.19)	-0.32 (0.18)	0.41 (0.12)	-0.20 (0.28)	-0.16 (0.22)	-0.05 (0.17

See Table I for trait description. All estimates from bivariate analyses; approximate standard errors in parentheses; semen quality was evaluated at 12, 18 and 24 months of age